IntelliCage as a tool for measuring mouse behavior – 20 years perspective

Kiryk, Anna ; Janusz, Artur ; Zglinicki, Bartosz ; Turkes, Emir ; Knapska, Ewelina ; Konopka, Witold ; Lipp, Hans-Peter ; Kaczmarek, Leszek

Abstract: Since the 1980s, we have witnessed the rapid development of genetically modified mouse models of human diseases. A large number of transgenic and knockout mice have been utilized in basic and applied research, including models of neurodegenerative and neuropsychiatric disorders. To assess the biological function of mutated genes, modern techniques are critical to detect changes in behavioral phenotypes. We review the IntelliCage, a high-throughput system that is used for behavioral screening and detailed analyses of complex behaviors in mice. The IntelliCage was introduced almost two decades ago and has been used in over 150 studies to assess both spontaneous and cognitive behaviors. We present a critical analysis of experimental data that have been generated using this device.

DOI: https://doi.org/10.1016/j.bbr.2020.112620

Behavioural Brain Research 388 (2020) 112620

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

IntelliCage as a tool for measuring mouse behavior – 20 years perspective

Anna Kiryk[a], Artur Janusz[a], Bartosz Zglinicki[a], Emir Turkes[b], Ewelina Knapska[c], Witold Konopka[a], Hans-Peter Lipp[d,e], Leszek Kaczmarek[c,*]

[a] Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[b] Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, Irving Medical Center, New York, NY, USA
[c] BRAINCITY, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[d] Institute of Anatomy, University of Zurich, Zurich, Switzerland
[e] Institute of Evolutionary Medicine, University of Zurich, Zurich, Switzerland

ARTICLE INFO

Keywords:
IntelliCage
Behavior
Mice
Transgenic models
Human disorders
Depression
Emotional tests
Ethological approach
Cognitive functions
Learning and memory

ABSTRACT

Since the 1980s, we have witnessed the rapid development of genetically modified mouse models of human diseases. A large number of transgenic and knockout mice have been utilized in basic and applied research, including models of neurodegenerative and neuropsychiatric disorders. To assess the biological function of mutated genes, modern techniques are critical to detect changes in behavioral phenotypes. We review the IntelliCage, a high-throughput system that is used for behavioral screening and detailed analyses of complex behaviors in mice. The IntelliCage was introduced almost two decades ago and has been used in over 150 studies to assess both spontaneous and cognitive behaviors. We present a critical analysis of experimental data that have been generated using this device.

1. Introduction

Over the past decades, a vast number of genetically modified mice that carry mutations of genes associated with the nervous system have been generated. They allow the assessment of gene-behavior relationships that reveal links between individual genes and complex behaviors, such as activity [1], anxiety [2], aggression [3], and learning and memory [4,5]. Mutant mice have become the animal model of choice to mimic specific human genetic conditions and various brain disorders. The need for high-throughput, well-standardized, and validated methods of behavioral screening have subsequently emerged.

Initially, mouse behavior was most often assessed using tasks that were primarily developed for rats. Unlike rats, however, adult mice are difficult to handle and habituate to human experimenters. Thus, introducing mice to test chambers causes considerable stress that can obscure results. Furthermore, isolating mice, either for easier handling or home cage testing, is a potential long-term stressor that can affect behavior [6]. Therefore, there is a pressing need to reduce variability that is caused by environmental factors, human handling, and poorly standardized housing and experimental protocols. In order to obtain standardized scoring methods at least three approaches have been applied by behavioral neuroscientists: (i) using fully automated equipment, such as open field, 0-maze, elevated plus maze, acoustic startle, prepulse inhibition, fear conditioning, operant learning or automated home cage-like systems: Phenomaster, Phenotyper, Phenocube, IntelliCage, (ii) semi-automated programs tracking mice in several mazes (Morris water maze, open field, novel object recognition, 3-chambered social tests), (iii) non-automated scoring based on researcher observation and skills used for forced swim, tail suspension, reciprocal social interactions, evaluation of self-grooming, etc.

Here we focus on the IntelliCage, a fully automated system for the behavioral assessment of mice that live in social groups [7,8]. Importantly, automation can improve reproducibility and thus decrease the number of animals and experimental replications that are required to obtain reliable results.

The IntelliCage was inspired by observations of freely moving mice in their natural environment, particularly genetically lesioned animals that were living in large outdoor pens and subjected to constantly changing environmental and social conditions [9–11]. Laboratory mice rapidly responded to restricted food supply received in computer-controlled complex feeder boxes in the outdoor pens, by adapting their temporal and spatial patterns of feeding to the new situation [9,10]. Such naturalistic experimental setup was then scaled down to a big cage in the laboratory, namely the IntelliCage. It provides a training/testing

* Corresponding author.
E-mail address: l.kaczmarek@nencki.edu.pl (L. Kaczmarek).

https://doi.org/10.1016/j.bbr.2020.112620
Received 7 February 2020; Accepted 23 March 2020
Available online 14 April 2020

Table 1
Current state of behavioral knowledge obtained with the IntelliCage by 80 research groups to date. Approximately 3500 animals have been investigated, including inbred and outbred strains of mice (C57BL/6, C3H, DBA/2, 129S2, BALB/c, and Swiss albino mice), wild housed mice, bank voles, long-tailored wood mice, and approximately 50 genetically modified mouse strains. Pharmacological treatment with drugs and interventional studies (e.g., irradiation and traumatic brain injury) have also been conducted.

Behavioral parameter	Procedure	Reference
ACTIVITY	Free exploration	Akbergenov 2018 [121]; Berry 2012 [47]; Branchi 2013 [75]; Cathomas 2015 [35], Cathomas 2015 [122]; Codita 2010 [22]; Codita 2012 [55]; Endo 2012 [66]; Ermakova 2011 [25]; Festa 2019 [123]; Fischer 2017 [124]; Gapp 2014 [61]; Gumucio 2013 [125]; Heidari 2016 [24]; Heinla 2018 [126]; Ishii 2015 [67]; Ismail 2017 [127]; Jedynak 2012 [14]; Jensen 2015 [84]; Kalm 2013 [49]; Kiryk 2008 [18]; Kobayashi 2013 [33]; Konopka 2010 [41]; Krackow 2010 [50]; Kulesskaya 2013 [12]; Kulesskaya 2014 [76]; Lan 2011 [44]; Lee 2015 [43]; Macpherson 2016 [128]; Masuda 2016 [87]; Mechan 2009 [58]; Mijakowska 2017 [129]; Milior 2015 [74]; Muthuraju 2012 [32]; Muthuraju 2013 [130]; Nowak 2013 [69]; Ogi 2013 [131]; Ogi 2015 [132]; Onishchenko 2007 [34]; Osman 2014 [59]; Patrikainen 2014 [71]; Pelsoczi and Levay 2017 [133]; Raab 2018 [134]; Radwanska and Kaczmarek 2012 [72]; Ramakers 2012 [57]; Robinson and Riedel 2014 [15]; Roccaro-Waldmeyer 2018 [135]; Roughton 2012 [51]; Rudenko 2009 [16]; Ryan 2013 [42]; Sato 2018 [104]; Schuler 2012 [136]; Simmons 2016 [137]; Too 2016 [26]; Too 2016 [138]; Too 2016 [139]; Too 2016 [36]; Too 2019 [119]; Ujita 2018 [140]; van Dijk 2016 [38]; van Dijk 2019 [141]; Vannoni 2014 [21]; Viosca 2009 [17]; Voikar 2010 [142]; Voikar 2018 [31]; Xia 2015 [56]; Yang 2016 [82]; Zheng 2018 [143]; Zhu 2010 [54]
	Habituation	Benner 2014 [65]; Berry 2012 [47]; Caly 2019 [144]; Codita 2010 [22]; Gumucio 2013 [125]; Harda 2018 [145]; Heidari 2016 [24]; Holter 2015 [146]; Kobayashi 2013 [33]; Krackow 2010 [50]; Maroteaux 2018 [147]; Mechan 2009 [58]; Onishchenko 2007 [34]; Perez-Alcazar 2014 [148]; Ramakers 2012 [57]; Rudenko 2009 [16]; Ryan 2013 [42]; Too 2014 [27]; Too 2014 [29]; Too 2014 [30]; Too 2019 [119]; vanDijk 2016 [38]; van Dijk 2019 [141]; Voikar 2013 [23]; Weyer 2011 [13]
	Nosepoke adaptation	Cathomas 2015 [35]; Cathomas 2015 [122]; Codita 2012 [55]; Ermakova 2011 [25]; Heidari 2016 [24]; Holter 2015 [146]; Ishii 2015 [67]; Ismail 2017 [127]; Kalm 2013 [49]; Krackow 2010 [50]; Kulesskaya 2013 [12]; Kulesskaya 2014 [76]; Lee 2015 [43]; Maroteaux 2018 [147]; Marwari and Dave 2019 [78]; Mechan 2009 [58]; Mijakowska 2017 [129]; Ogi 2013 [131]; Ogi 2015 [132]; Raab 2018 [134]; Sano 2016 [149]; Serchov 2020 [150]; Simmons 2016 [137]; Too [26,27,28,29,36,138,139]; Weyer 2011 [13]; Xia 2015 [56]; Yang 2016 [82]; Zhu 2010 [54]
	Long-term home cage activity	Rudenko 2009 [16]; Rudenko 2019 [151]
	Circadian activity	Cathomas 2015 [35]; Cathomas 2017 [152]; Hardt 2019 [153]; Heinla 2018 [126]; Kulesskaya 2014 [76]; Marwari and Dave 2018 [154] and 2019 [78]; Mohammadi 2017 [155]; Piechota 2012 [156]; Too [26,119,138]; Ujita 2018 [140]; Welz 2019 [157]
EMOTIONALITY	Reaction to new environment	Ben Abdallah 2013 [158]; Galsworthy 2005 [20]; Krackow 2010 [50]; Kulesskaya [12,76]; Onishchenko 2007 [34]; Peltola 2015 [159]; Rudenko 2009 [151]; Simmons 2016 [137]; Ujita 2018 [140]; Voikar 2013 [23]
	Novel object preference	Codita 2010 [22]; Faizi 2011 [48]; Mechan 2009 [58]
	Novel smell (neophobia)	Codita 2010 [22]
	Light response test	Too [26,27,28,29,30,36,119,139]
LEARNING and MEMORY	Drinking session adaptation	Codita 2010 [22]; Festa 2019 [123]; Frohlich 2019 [160]; Ishii 2015 [67]; Kobayashi 2013 [33]; Kulesskaya 2014 [76]; Lee 2015 [43]; Maroteaux 2018 [147]; Safi 2006 [83]; Too [26,36,138,139]; van Dijk 2019 [141]; Voikar 2018 [31]
	Place learning	Albuquerque 2013 [161]; Barlind 2010 [46]; Ben Abdallah 2013 [158]; Berry 2012 [47]; Caly 2019 [144]; Codita [22,55]; Dere 2018 [162]; Ermakova 2011 [25]; Faizi 2011 [48]; Fischer 2017 [124]; Fuchs 2018 [163]; Galsworthy 2005 [20]; Gumucio 2013 [125]; Hardt 2019 [153]; Heidari 2016 [24]; Holter 2015 [146]; Huo 2012 [40]; Ismail 2017 [127]; Itawa 2014 [164]; Jaholkowski 2009 [45]; Jensen 2019 [165]; Kalm [49,60]; Karlsson 2011 [19]; Kato 2018 [102]; Kiryk 2008 [18]; Knapska [53,73]; Kobayashi 2013 [33]; Konopka 2010 [41]; Koss 2016 [166]; Krackow 2010 [50]; Kulesskaya [12,76]; Lan 2011 [44]; Lee 2015 [43]; Maroteaux 2018; [147]; Masuda 2016 [87]; Matlik 2018 [167]; Mechan 2009 [58]; Netrakanti 2015 [168]; Onishchenko 2007 [34]; Orock 2018 [169]; Osman 2014 [59]; Pan 2018 [170]; Pelsoczi and Levay 2017 [133]; Peltola 2015 [159]; Perez-Alcazar 2014 [148]; Puscian 2014 [100]; Raab 2018 [134]; Robinson 2014 [15]; Roccaro-Waldmeyer 2018 [135]; Roughton 2012 [51]; Rudenko 2009 [16]; Ryan 2013 [42]; Sato 2018 [104]; Sekiguchi 2011 [171]; Simmons 2016 [137]; Ujita 2018 [140]; Vazquez 2015 [81]; Voikar 2018 [31]; Vyssotski 2011 [172]; Weyer 2011 [13]; Wilhelmsson 2019 [106]; Xia 2015 [56]; Yang 2016 [82]; Zheng 2018 [143]; Zhu 2010 [54]
	Reversal place learning	Albuquerque 2013 [161]; Ben Abdallah 2013 [158]; Berry 2012 [47]; Codita [22,55]; Dere 2018 [162]; Fischer 2017 [124]; Fuchs 2018 [163]; Galsworthy 2005 [20]; Gumucio 2013 [125]; Hardt 2019 [153]; Heidari 2016 [24]; Holter 2015 [146]; Huo 2012 [40]; Ismail 2017 [127]; Itawa 2014 [164]; Jensen 2019 [165]; Kalm [49,60]; Karlsson 2011 [19]; Kato 2018 [102]; Kobayashi 2013 [33]; Koss 2016 [166]; Krackow 2010 [50]; Kulesskaya [12,76]; Lan 2011 [44]; Lee 2015 [43]; Maroteaux 2018; [147]; Masuda 2016 [87]; Mechan 2009 [58]; Netrakanti 2015 [168]; Onishchenko 2007 [34]; Orock 2018 [169]; Osman 2014 [59]; Pan 2018 [170]; Pelsoczi and Levay 2017 [133]; Peltola 2015 [159]; Perez-Alcazar 2014 [148]; Puscian 2014 [100]; Raab 2018 [134]; Robinson 2014 [15]; Roccaro-Waldmeyer 2018 [135]; Roughton 2012 [51]; Rudenko 2009 [16]; Ryan 2013 [42]; Sato 2018 [104]; Sekiguchi 2011 [171]; Simmons 2016 [137]; Ujita 2018 [140]; Vazquez 2015 [81]; Voikar 2018 [31]; Weyer 2011 [13]; Wilhelmsson 2019 [105,106]; Xia 2015 [56]; Yang 2016 [82]; Zhu 2010 [54]

(*continued on next page*)

Table 1 (*continued*)

Behavioral parameter	Procedure	Reference
	Serial Reversal place learning	Akbergenov 2018 [121]; Huo 2012 [40]; Kalm 2013 [49]; Karlsson 2011 [19]; Masuda 2018 [173]; Matlik 2018 [167]; Osman 2014 [59]; Roughton 2012 [51]
	Extinction of place preference	Codita 2010 [22]; Gumucio 2013 [125]; Wilhelmsson 2019 [105]
	Side learning/discrimination	Cathomas 2015 [122]; Codita 2012 [55]; Knapska 2013 [73]; Marwari and Dave 2018 [154]; Osman 2014 [59]; Rudenko 2009 [16]; Serchov 2020 [150]; Voikar 2018 [31]
	Reversal Side learning	Codita 2012 [55]
	Place avoidance	Albuquerque 2013 [161]; Codita 2010 [22]; de Hoz 2018 [174]; d'Isa 2011 [175]; Faizi 2011 [48]; Gumucio 2013 [125]; Hardt 2019 [153]; Itawa 2014 [164]; Jaholkowski 2009 [45]; Jensen 2015 [84]; Karlsson 2011 [19]; Kiryk 2008 [18]; Knapska [53,73]; Kobayashi 2013 [33]; Marwari and Dave 2018 [154]; Masuda 2016 [87]; Mechan 2009 [58]; Nowak 2013 [69]; Rudenko 2009 [16]; Voikar [31,142]
	Reversal of place avoidance	Mechan 2009 [58]; Voikar 2018 [31]
	Extinction of place avoidance	d'Isa 2011 [175]; Hardt 2019 [153]; Itawa 2014 [164]; Masuda 2016 [87]; Nowak 2013 [69]; Voikar 2010 [142]
	Cued punishment test	Lan 2011 [44]
	Sequencing task	Aung 2016 [176]; Benner 2014 [65]; Endo [62,66]; Gapp 2014 [61]; Hardt 2019 [153]; Macpherson 2016 [128]; Marwari and Dave [78,154]; Sano 2016 [149]
	Patrolling (e.g.clockwise)	Akbergenov 2018 [121]; Albuquerque 2013 [161]; Fischer 2017 [124]; Holter 2015 [146]; Kobayashi 2013 [33]; Kulesskaya 2014 [76]; Matlik 2018 [167]; Onishchenko 2007 [34]; Peltola 2015 [159]; Rudenko 2009 [16]; Too [26,36,138,139]; van Dijk 2019 [141]; Vazquez 2015 [81]; Voikar 2018 [31]; Weyer 2011 [13]; Zheng 2018 [143]
	Patrolling reversal	Albuquerque 2013 [161]; Kobayashi 2013 [33]; Too [26,36,138,139]; van Dijk 2019 [141]; Voikar 2018 [31]
	Chaining	Akbergenov 2018 [121]; Fischer 2017 [124]; Kobayashi 2013 [33]; Matlik 2018 [167]
	Reaction time task / motor impulsivity	Fischer 2017 [124]; Kobayashi 2013 [33]; Masuda [87,173]; Matlik 2018 [167]; van Dijk 2016 [38]
	Delay discounting task	Gapp 2014 [61]; Hardt 2017 [177]; Kato 2018 [102]; Masuda [87,173]; Matlik 2018 [167]; Ruud 2019 [178]
	DRL (differential reinforcement of lower rates) paradigm	Atlan 2018 [179]; Gapp 2014 [61]; Hardt 2017 [177]; Fischer 2017 [124]; Kobayashi 2013 [33]; van Dijk 2016 [38]
	Conditioned drinking suppression	Voikar 2010 [142]
	LED stimulus-dependent alternation	Voikar 2018 [31]
	Probabilistic choice procedure	Jablonska 2019 [180]; Ruud 2019 [178]
SOCIAL BEHAVIORS	Group place learning	Galsworthy 2005 [20]; Harda 2018 [145]; Jaholkowski 2009 [45]; Kiryk 2011 [52]
	Competition task	Benner 2014 [65]; Benner 2015 [181]; Endo 2012 [66]; Ishii 2015 [67]; Kulesskaya 2013 [12]; Ujita 2018 [140]
	Social interactions in alcohol drinking	Smutek 2014 [64]
	Social modulation of aversive memories	Nowak 2013 [69]
	Social stress	Branchi 2010 [70]; Branchi 2013 [98]; Bergamini 2016 [182]
OTHER BEHAVIORS in PHARMACOLOGICAL TREATMENT	Different tests for alcohol drinking	Beroun 2018 [183]; Holgate 2017 [184]; Jablonska 2019 [180]; Koskela 2018 [185]; Mijakowska 2017 [129]; Parkitna 2013 [77]; Radwanska 2012 [72]; Smutek 2014 [64]; Stefaniuk 2017 [90]
	Vogel water-lick paradigm adapted to IC	Fischer 2017 [124]; Safi 2006 [83]; van Dijk 2016 [38]
	Fixed/Progressive ratio (operant conditioning response)	Jastrzebska 2016 [186]; Poggini 2019 [103]; Serchov 2020 [150]; Skupio 2017 [187]; Vazquez 2015 [81]
	Chronic unpredictable stress/ Acute stress	Alboni 2015 [79]; Alboni 2016 [188]; Branchi 2013 [75]; Jensen 2015 [84]; Milior 2015 [74]; Poggini 2019 [103]
	Anhedonia / taste aversion	Alboni 2015 [79]; Alboni 2016 [188]; Bergamini 2016 [182]; Branchi 2010 [70]; Branchi 2013 [75]; Dere 2018 [162]; Heinla 2018 [126]; Matlik 2018 [167]; Milior 2015 [74]; Mohammadi 2017 [155]; Poggini 2019 [103]; Serchov 2020 [150] / Ratner 2016 [189]
	Cocaine/Morphine self-administration	Ajonijebu 2018 [190]; Ajonijebu 2019 [80]; Skupio 2017 [187]

system to measure spontaneous activity, emotional responses, discrimination learning, spatial memory, and operant conditioning in mice that live in relatively low-stress conditions without handling or social isolation. To date, the IntelliCage has been used to phenotype various mouse models that have been reported in more than 150 publications (Table 1). The data that have been collected over the years allow for a critical review and assessment of the IntelliCage system, which we present below.

2. IntelliCage system set-up

The IntelliCage is a large home cage that has four computer-controlled corners, called learning or recording corners. Learning corners can be visited when a mouse passes a tubular antenna that reads the code of an implanted radio-frequency identification (RFID) chip (Fig. 1). An animal that visits a corner faces two operant conditioning walls (left and right), each equipped with three light-emitting diodes (LEDs) that deliver different visual stimuli. Access to the nipple of bottles is provided through a hole with built-in nosepoke sensors. Mice can be supplied with tap water, sweetened water, bitter water, sour water or liquid with diluted drugs. Lickometers measure the number of occurrences and time of contact with the drinking nipple. Sliding doors that are operated by the computer can open or block access to water without harming the mice. The corners have a space for just one mouse;

thus, only one animal can "work" in a corner at a given time, and its presence is detected by an infrared sensor. Aversive stimuli can also be delivered in the form of air-puffs. Up to 16 mice can be kept in the cage without having to change the bedding for 1 week. All visits, nosepokes, and licks are recorded and transferred to a controlling computer that also stores the output actions of the system, such as door openings, air-puffs, and LED activation. The system is controlled by software that offers a graphic interface and permits the programming of a wide range of behavioral tests, from simple activity monitoring to delay-discounting operant conditioning. Individual and averaged behaviors are monitored on a screen. The data are stored on a disk and can be analyzed off-line, leaving the original data untouched.

3. Basal activity in mice assessed in the IntelliCage

Basal activity is the main parameter of an animal's health, emotional state, and well-being. Classical exploratory behavior tests, such as open field and elevated plus maze, measure forced rather than unrestricted behavior. Conversely, home cage-like conditions in the IntelliCage provide a unique experimental opportunity to assess activity freely initiated by animals. Additionally, short- and long-term habituation and circadian rhythm can be recorded.

3.1. Comparison of the IntelliCage with standard tests

Numerous studies have compared exploration in the IntelliCage to activity in open field and elevated plus maze and movement sensor-equipped home cage paradigms. Similar changes in activity were observed in standard tests and the IntelliCage in six different mouse models [12–17]. However, normal activity in the IntelliCage contradicted aberrant activity in standard tests in GLT1$^{+/-}$ mice [18], possibly reflecting the effects of handling-induced stress in non IntelliCage approaches. Additionally, irradiated C57BL/6 mice exhibited alterations of activity during three 5-day sessions in the IntelliCage that were not detected in a 50-min open field test in the same mice [19]. The results show that some aspects of treatment-induced changes in the activity are detected in standard tests and others in the IntelliCage.

3.2. Adaptation, habituation and patrolling in the IntelliCage

In the IntelliCage, the main measure of activity is visits to corners. During the first 12 h after introduction to the IntelliCage, mice intensely explore the novel environment [20], which helps them acquire spatial information and become familiar with the relatively large complex cage [16]. Adaptation to the cage involves learning and is usually achieved before the second day after initiating the experiment [21]. However, in a mouse model of Alzheimer's disease, the habituation of 14-month-old mutant mice lasted longer than 2 days [22]. Moreover, during the first days of housing in the IntelliCage, other abnormalities in exploratory activity and habituation were detected in various mouse models [13,16,22–30].

Multivariate analyses of spontaneous behavior in the IntelliCage have found, by means of canonical discriminant analysis, that regularity in patrolling patterns can discriminate mice with hippocampal lesions from controls and animals with prefrontal lesions [31]. Likewise, this type of analysis is able to discriminate statistically diverse age groups of mice that are housed in the same cage (T. Endo, personal communication). The IntelliCage has also been used to measure post-trauma recovery and the restoration of normal locomotor activity after brain injury [32].

Living in groups does not affect spontaneous activity of individual mouse in the IntelliCage. Deficits in adaptation, habituation and patrolling patterns have been detected in at least 15 mouse models compared to controls.

3.3. Circadian activity

Circadian activity is a parameter that is difficult to monitor in standard behavioral tests. Data that have been generated across different studies show that after habituation to a new cage, mice develop a stable circadian rhythm of activity (Fig. 2). The IntelliCage also allows the detection of sleep/activity impairments in genetically modified or drug-treated mice. Persistent hyperactivity [33], hypoactivity [15,34], atypically high activity during the inactive period [35], and abnormal circadian activity [29,36] have been reported in different mouse models. The number of patrol visits was shown to decrease with age, but drinking activity remained unchanged in FVB/N mice up to 18 months of age (unpublished results).

The permanent recording of circadian activity permits a simple test to assess hippocampal deficits in sensing time. Access to water is restricted to time slots. Normal mice exhibit an increase in activity that is synchronized with drinking time, whereas chronic hippocampal lesions are characterized by ill-timed bouts of activity [31] (Fig. 3).

3.4. Neophobia and anxiety

Basal activity in mice can be modified by their emotional state. Neophobia and anxiety are measures of emotionality that can be assessed immediately after introducing animals to a new, complex environment, such as the IntelliCage. For example, the latency to the first visit to a corner of the IntelliCage was prolonged in mice with hippocampal lesions [37] and in mice that were treated with methylmercury [34], which is consistent with the higher level of anxiety that is observed in these animals. Other measures of anxiety include the latency to the first operant response (i.e., nosepoke in a corner [23]; and the latency to the first drinking episode [16,38]). Mouse models of Huntington's disease also exhibited a longer latency to finding the source of water [16].

Emotional reactions to novelty can also be measured in familiar environments (after the habituation period) when circadian activity is stable. Exposure to new objects and visual/olfactory stimuli in learning corners may reveal novelty preference or avoidance. For example, Codita et al. [22] reported a significant increase in the number of visits to a corner with a new object. Similarly, the number of nosepokes was higher for bottles that contained scented water compared with tap water [22]. An increase in exploration of a familiar environment, such as the IntelliCage, in response to novelty exposure is consistent with results that show that low levels of anxiety facilitate exploration and reduce neophobia [39]. However, in a mouse model of pneumococcal meningitis, long-term photophobia was observed in response to a sequence of red, blue, and green lights that were activated by visits to a corner [30].

4. Cognitive functions detectable in the IntelliCage

Another application of the IntelliCage system is the development of various learning and memory-related tasks. To date, more than 40 protocols have been developed and evaluated by approximately 80 research groups (Table 1). Testing cognitive functions in the same familiar environment where animals are housed using the IntelliCage eliminates such confounding factors as contact with human experimenters and social isolation. Unlimited mouse activity and voluntary behaviors have been shown to allow rapid spatial learning, particularly in tests that measure preference for or avoidance of the learning corner. These qualities remain in opposition to standard tests, in which a single animal is observed within a short period of time and under significantly different conditions than in the home cage.

4.1. Comparison of learning protocols in the IntelliCage with standard tests

In most cases, consistent results between the IntelliCage system and

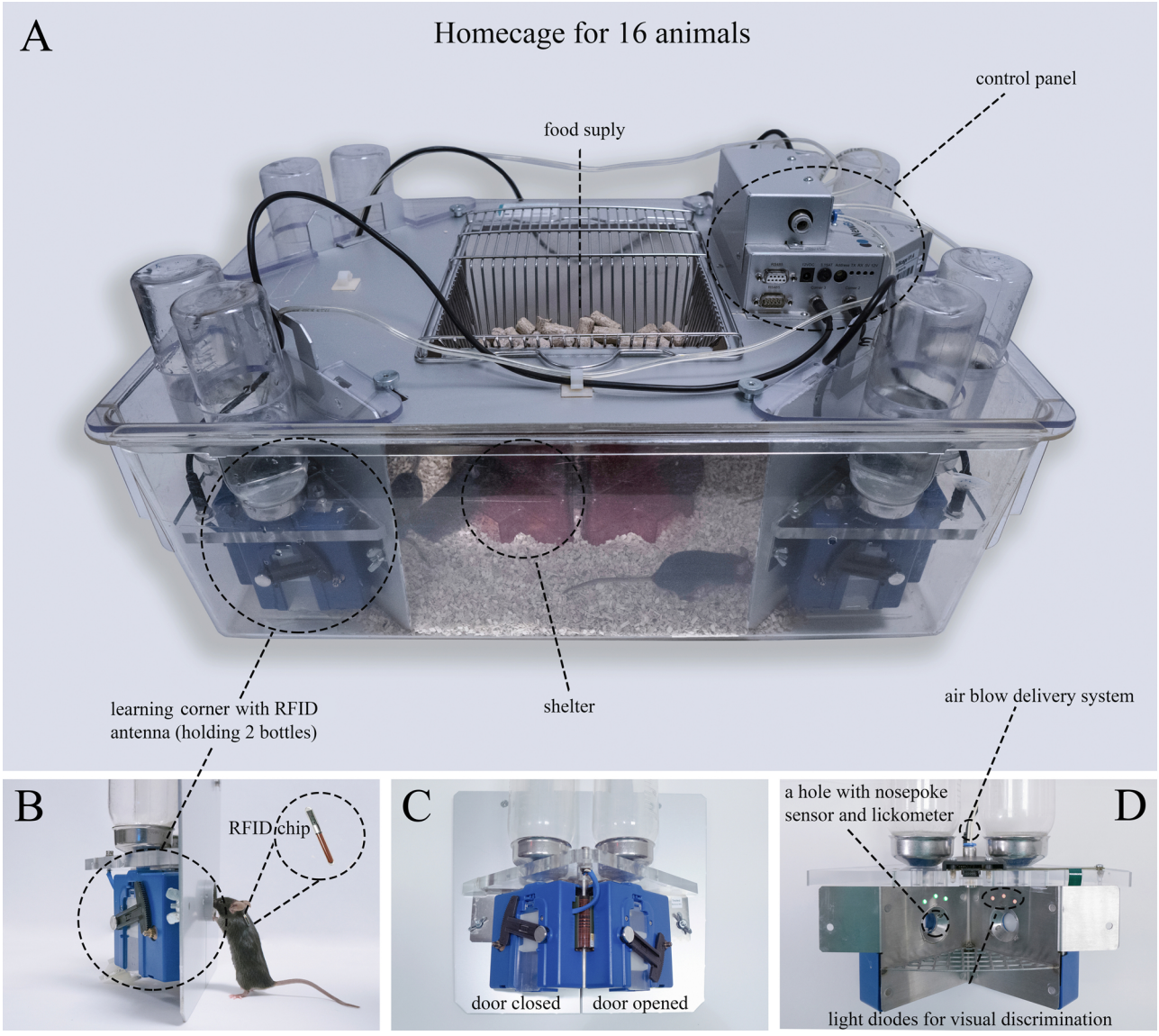
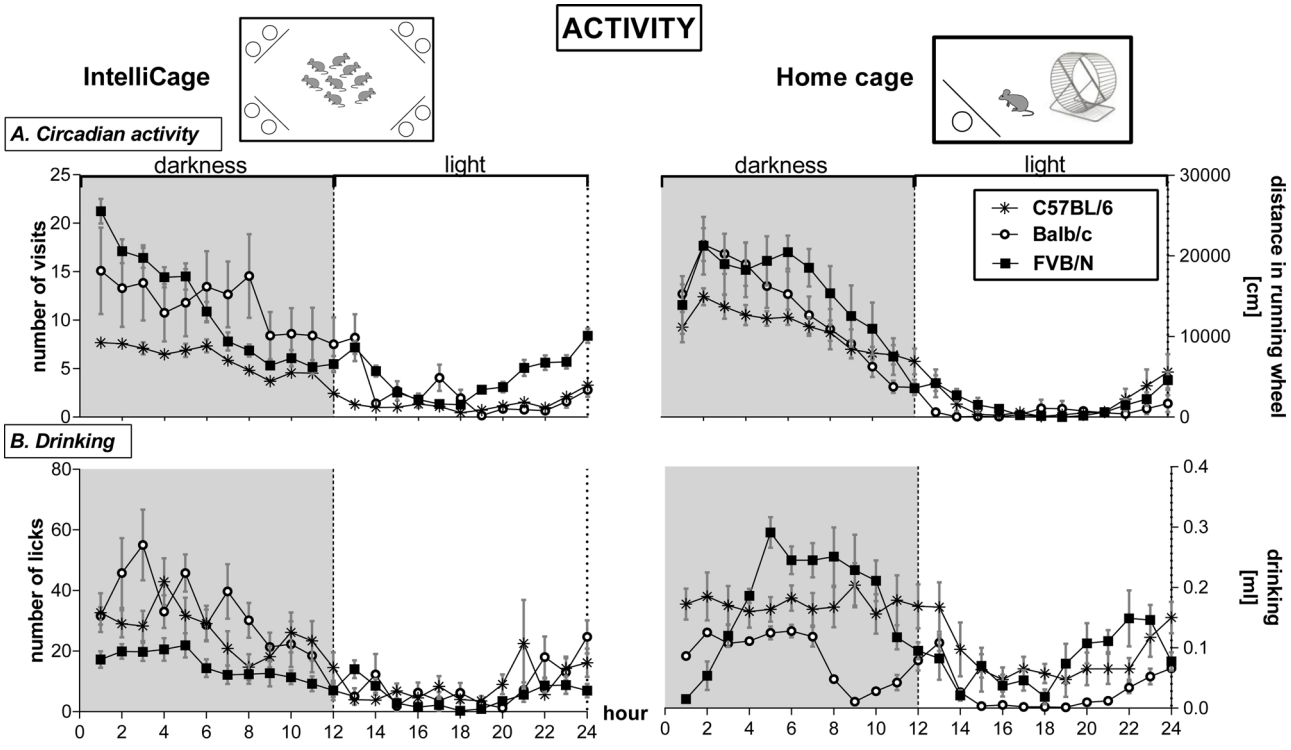


Fig. 1. IntelliCage setup. A. In a large cage (Tecniplast 2000), a group of mice have access to shelters, *ad libitum* food from the lid of the cage, and water from four learning corners. **B**. Testing of individual mice under conditions of social housing is possible by tagging each mouse with an RFID microchip. The mice are recognized when they pass a tubular antenna to enter the learning corner. Additionally, an infrared sensor on top of the corner compartment confirms the presence of the animal, which is then recorded as a corner entry. The presence of an individual mouse in the given corner blocks access for the other mice. **C**. **D**. Each learning corner contains two symmetrical operant conditioning units. Their walls contain an array of three LEDs that can be programmed to display different colors and patterns. Rewards (e.g., water, sucrose, and addictive drugs) can be obtained from the nipples of two bottles. Such a setup also allows for gustatory discrimination learning. Mice can reach the bottles through a hole that contains a photobeam, the crossing of which is recorded as a nosepoke. Access to the bottles can be gated by the programmed procedure, defined by the sequence of nosepokes. Licking the nipple of the bottle is recorded as a lick, the number and duration of which are acquired. Negative reinforcement can be delivered through an air-puff from the top of the learning corner. **D**. Access to a liquid reward can be allowed or blocked by motorized sliding doors.

standard tests have been obtained, specifically in various learning tasks in which either memory improvements or impairments were detected. Genetic mutations that are related to improvements in learning have been observed in both the IntelliCage and several standard tests, including the Morris water maze (Fig. 4), context-dependent fear conditioning [40], and trace fear conditioning [41]. Notably, Ryan et al. [42] and Lee et al. [43] reported that the IntelliCage system was as sensitive as the water maze in detecting spatial learning deficits in the PLB1^{Triple} knock-in mouse model of Alzheimer's disease and cyclic adenosine monophosphate-guanine nucleotide exchange factor (cAMP-GEF) knockout mice, respectively. Memory impairments were also observed in the IntelliCage and confirmed in the Morris water maze in APP.V717I mice (Fig. 4) and male mice that were exposed to chronic, sub-lethal hypoxia [44]. Proper procedural learning that was observed in the IntelliCage in other experimental models was confirmed by the learning of context, cue, and trace fear conditioning, novel object recognition [45] and performance in the Morris water maze [34].

4.2. Place learning

The most frequently used learning protocol in the IntelliCage system is spatial learning with appetitive reinforcement, referred to herein as simply place learning. In this task, an animal is expected to find a reward in one of the four corners of the IntelliCage. A reward can be either tap water [22,34,46–51] or sweetened water [18,19,41,45,52–54]. Preference for the correct corner develops very quickly. Within the first 24 h of learning above chance level (i.e., 25 % of all visits), an increase in the number of correct spatial responses was



Parameter	Behavioral test	Strain	Statistical test	Results	P value
A.	IntelliCage -	Balb/c		F(23,207)=16.74	P<0.001
CIRCADIAN	number of visits	C57BL/6	One-way ANOVA	F(23,1081)=57.58	P<0.001
ACTIVITY		FVB/N	with repeated	F(23,414)=54.78	P<0.001
	Home cage -	Balb/c	measures,	F(23,414)=7.19	P<0.001
	number of rotations	C57BL/6	time effect:	F(23,161)=20.23	P<0.001
	in running wheel (cm)	FVB/N		F(23,184)=15.62	P<0.001

Parameter	Behavioral test	Strain	Statistical test	Results	P value
B.	IntelliCage -	Balb/c		F(23,184)=7,79	P<0.001
DRINKING	number of licks	C57BL/6	One-way ANOVA	F(23,713)=9,92	P<0.001
		FVB/N	with repeated	F(23,414)=7,19	P<0.001
	Home cage -	Balb/c	measures,	F(23,161)=9.08	P<0.001
	drinking (ml)	C57BL/6	time effect:	F(23,161)=3.04	P<0.001
		FVB/N		F(23,69)=33.08	P<0.001

Fig. 2. Changes in circadian activity in the IntelliCage are similar to cages with a running wheel for three inbred strains. C57Bl/6 (n = 12), Balb/c (n = 10), and FVB/N (n = 5) mice were studied. Each time point shows the average value of a few days of the experiment (4-7 days). **A**. (Left) As measured in the IntelliCage, the number of visits to the drinking corners during the active phase (0-12 h, dark phase) is higher than during the inactive phase (12-24 h, light phase). Mice reach the peak of exploration in the dark phase, decreasing to a minimum in the light phase. (Right) In home cages with running wheels, mice exhibit a similar activity profile. **B**. Mice drink more in the dark phase than in the light phase, regardless of the housing conditions (see also [16,21,22]).

observed [18,19,34,40,41,45,47–49,51,55–58]. Memory of the rewarded corner persists even when mice are removed from the IntelliCage. For example, mice that were trained to choose one of the corners and then were removed from the IntelliCage for 72 h still preferred the previously rewarded corner after reintroduction to the IntelliCage [48].

Poor place preference learning, reflected by a longer time spent in non-rewarded corners [19,40,49,51,59] or repeated unsuccessful attempts to open incorrect doors [49], has been observed in several mouse models, such as PLB1^{Triple} knock-in mice (i.e., an animal model of Alzheimer's disease) [22] and irradiated C57BL/6 mice [59,60]. In some cases, better place learning has been reported, such as in irradiated mice that were treated with lithium [40], irradiated mice that lacked the third complement component [60], and neuron-specific Dicer knockout mice [41].

4.3. Complex cognitive tests

More complex learning and memory procedures that measure either goal-directed behaviors or behavioral flexibility have also been performed using the IntelliCage. Gapp et al. [61] observed superior performance in a delay-discounting task in the IntelliCage compared with differential reinforcement of lower rates of responding (DRL) in a conventional task in the offspring of mice that were exposed to traumatic stress in postnatal life. Furthermore, deficits in spatial working memory in amyloid precursor protein double mutant (APPsα-DM) mice in the T-maze and radial maze were accompanied by deficits in the patrolling task in the IntelliCage. In this test, mice had to remember the last corner where they obtained a reward to learn the correct response pattern [13].

A cognitively interesting variant is rule learning, which is based on a serial reversal task as presented by Endo et al. [62] (Fig. 5). The task models a simple human test to detect impairments in executive function [63]. Mice must learn to obtain water during a limited time window by shuttling diagonally between two corners that are assigned by the program. After 5–7 days, the corners are changed, and the mice have to relearn the alternative diagonal shuttling route. This procedure is repeated 3–4 times. After a reversal, mice usually commit a high number of errors, but these error peaks eventually decline, indicating that the mice learned the reversal rule. Consistent results were reported in parallel studies that were conducted in Japan and Switzerland. However, this task is rather time-consuming and thus unsuitable for high-throughput testing. Recently, a novel variant of the test was designed by Endo and Benner (personal communication), in which mice must reach a certain criterion of correct responses before the diagonal corridor is switched. Intriguingly, very old mice appear to solve the rule learning as good as or better than young mice. This implies that IntelliCage protocols can be used to assess cognitive reserve in elderly mice that are relatively fragile and exhibit impairments in sensory capacities but retain substantial cognitive abilities.

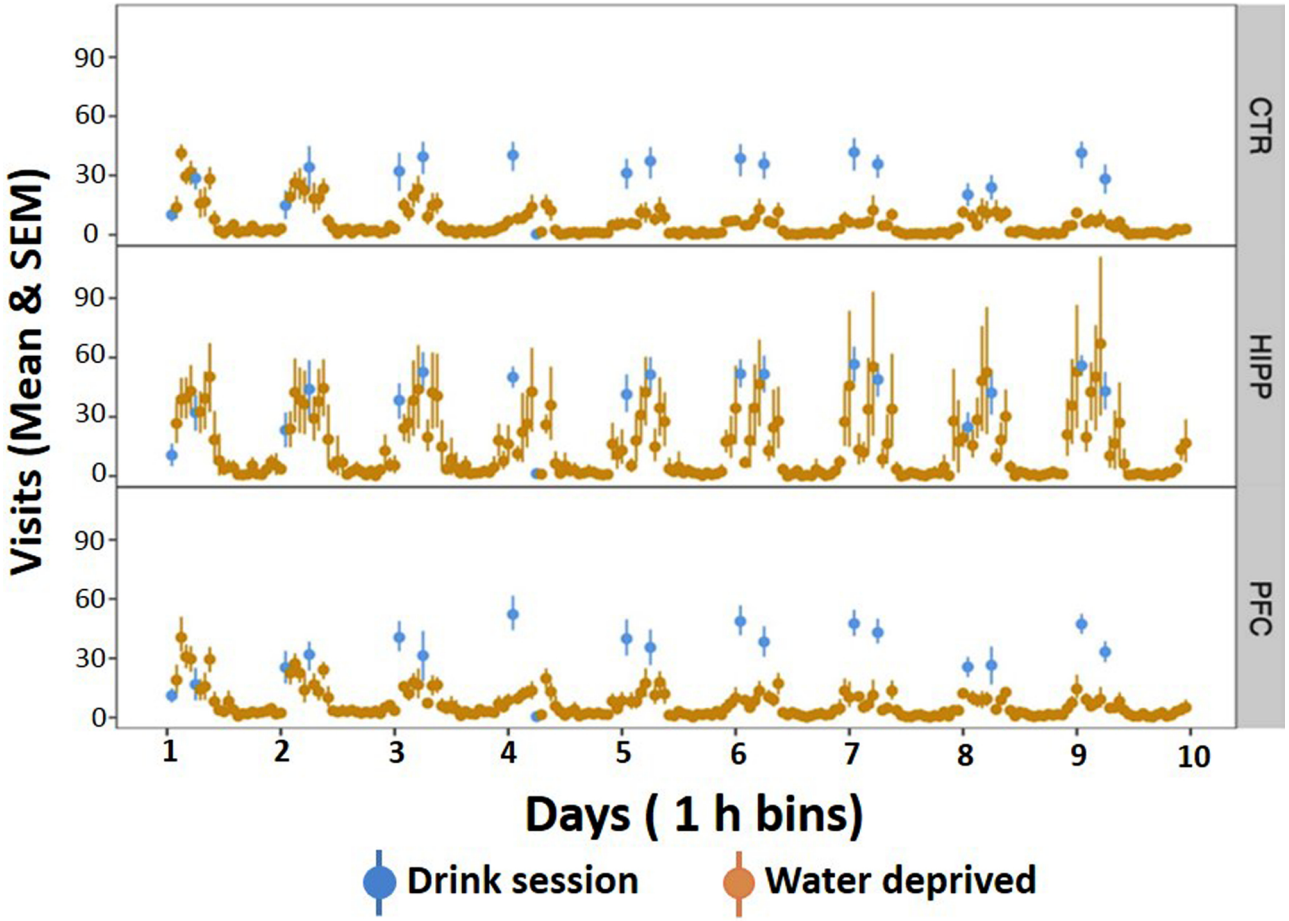


Fig. 3. Analysis of hippocampal malfunction in sensing time. Three groups of mice were housed in the IntelliCages: control (CTR), hippocampal lesions (HIPP) and prefrontal lesions (PFC). Water was accessible only during two time slots of 1 h, separated by 3 h. Blue dots indicate that CTR and PFC mice were synchronizing their corner visits rather precisely within the time slots giving access to water, while HIPP mice appeared to be hampered in synchronizing their activity to the relevant time slots. On the fourth day, there was a time slot blocking access to water due to a technical error. Modified after Voikar et al. [31].

5. Group-specific behaviors and social interactions in the IntelliCage

Exploring social phenomena is demanding under standard conditions that analyze the behavior of an individual animal. To some extent, the IntelliCage system allows the assessment of group structure that is established during housing. Attempts have been made to identify clusters of corner entries by specific groups of animals (e.g., following a leader or mouse that previously entered the corner, e.g. Smutek et al. [64]).

Experimental and control mice are usually housed together in the IntelliCage in mixed groups to eliminate the impact of micro-environmental variations between cages. However, a potential confounding factor can be introduced when handicapped animals learn from control animals how to solve a task. Although this is rarely a problem, in cases in which no significant within-cage group differences are evident, the studied groups can be tested in separate IntelliCages.

5.1. Group place learning

In a task in which groups of mice have access to the same corner with reward, their cognitive ability was shown to be possibly modulated by the social context [52]. When transgenic mice with the London mutation of the human amyloid precursor protein (APP.V717I) were housed separately from control mice, APP.V717I mice were unable to develop place preference, whereas wildtype mice displayed a 55% preference for the rewarded corner (Fig. 6A, Separated). When mice of both genotypes were housed in the same cage, the entire group (both wild type and transgenic mice) developed a 40% preference for the rewarded corner (Fig. 6B, Mixed). The authors concluded that learning in APP transgenic mice improved when they were co-housed with healthy individuals.

5.2. Competitive dominance behavior

Other social behaviors that are measured in the IntelliCage include competitive dominance and subordination. Normally, the permanent availability of four corners per cage limits competition in the IntelliCage. Male competition can be induced by restricting access to the corner with water to 3 h/day [65–67], resulting in more visits. However, such competitive behavior can be suppressed by different harmful factors, such as a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [66], focal neuronal heterotopia [67], and early social isolation [65]. In the last study, the housing of 14 animals per cage was postulated as a highly competitive condition, and males from experimental group, but not females, were subordinate to control littermates when competing for reward access [65]. However, in different mouse model of CD73 knockouts with eight females per cage, wildtype mice presented dominance in the IntelliCage by spending more time in the rewarded corner than CD73 knockouts. A battery of other standard social tests confirmed these IntelliCage findings [12].

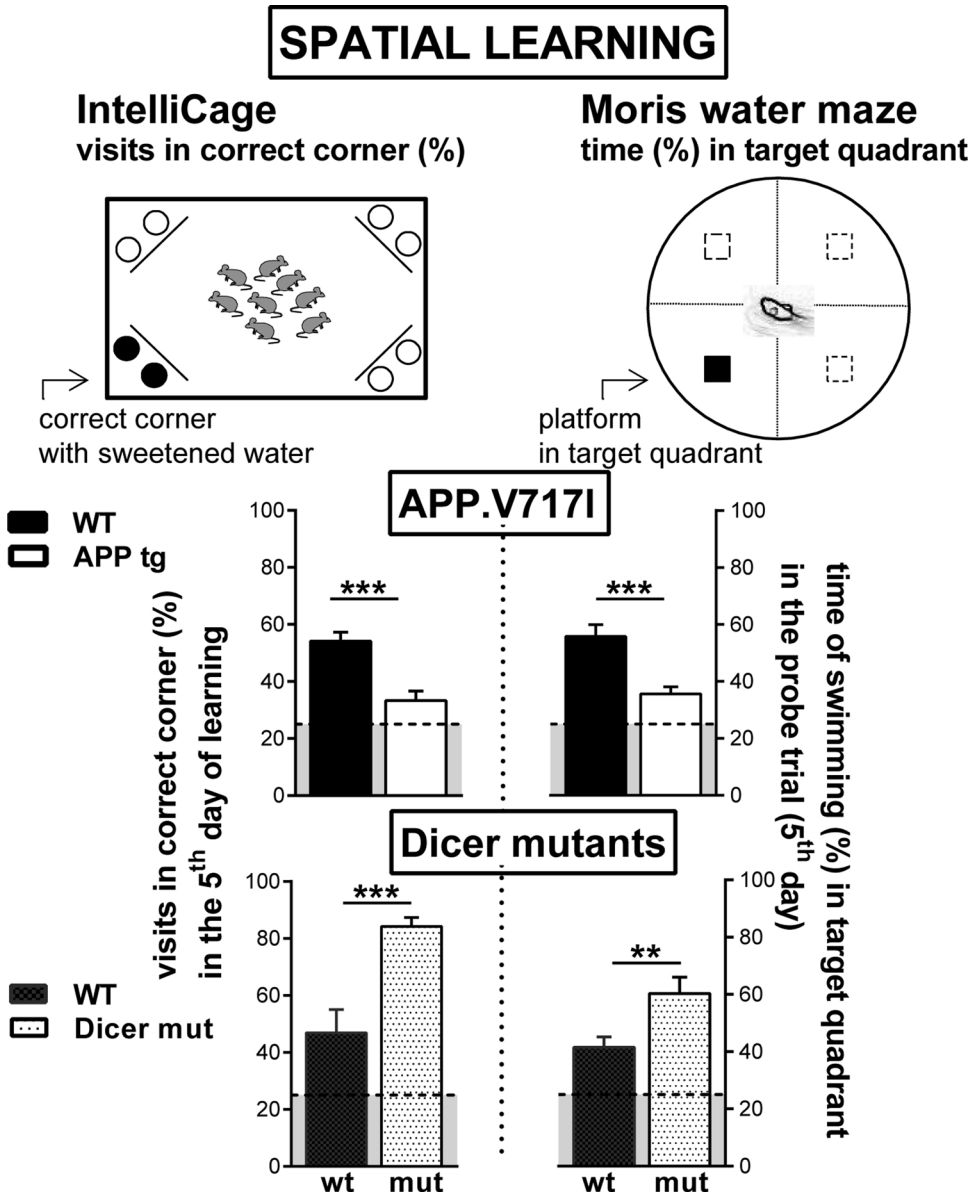


Fig. 4. Learning tests in the IntelliCage and Morris water maze detect parallel changes in mutant mice. In the place learning task in the IntelliCage, to drink water, a mouse must find and remember one corner that can be opened by a nosepoke. The correct corner is different for each mouse. The doors in the remaining corners are permanently closed, blocking access to the bottles. All of the control mice (black bars) developed a preference for the correct corner by performing more than 40 % of visits to this corner by day 5 of learning. APP.V717 l mutants (top) exhibited a deficit, whereas Dicer mutants (bottom) exhibited an increase in cognitive ability in the place learning task in the IntelliCage. In the Morris water maze task, the spatial memory of a platform position was tested in the probe trial (day 5). All of the control mice (black bars) searched for the platform in the proper quadrant of the pool for approximately 40 % of the total swimming time. Again, APP.V717 l and Dicer mutants exhibited poorer and better performance, respectively. $^{**}p < 0.01$, $^{***}p < 0.001$.

5.3. Social modulation of aversive memories

Social learning in mice has been shown to be an adaptive behavior that allows the generation of responses based on the experiences of others [68]. Adaptive behavior that is based on the responses of other group members has also been observed in the IntelliCage. The return of fear after extinction, guided by a fearful cage-mate, was observed in parallel in classic fear conditioning procedures and in the IntelliCage [69].

6. The IntelliCage in pharmacological and toxicological studies

To evaluate whether a drug influences activity, emotionality, or learning, mice have been exposed to the substance either acutely or chronically. Monitoring the effects of drugs requires a battery of standard tests, but the IntelliCage meets both criteria (i.e., acute, simultaneous drug testing in a group of animals and long-term evaluations of the effects of drugs on behavior).

6.1. Examples of compounds tested in the IntelliCage

The evaluation of substances in the IntelliCage is usually achieved by dissolving them in water and delivering them to mice in a total of eight bottles. To date, mice have been exposed to sucrose [18,19,41,45,52,53,70–73], saccharin [35,61,74–76], fructose [54], NaCl and citric acid [71], quinine [64,71,73], alcohol [64,72,77], haloperidol [78], fluoxetine [79], cocaine [80] in the IntelliCage. Spontaneous and free-choice behaviors have been shown to lead to either preference for or avoidance of the corner where the solution of a compound is located. An experimental diet that contained the human milk oligosaccharide 2′-fucosyllactose was given to mice in the IntelliCage and was associated with improvements in learning in a fixed-ratio operant conditioning task [81].

The IntelliCage has been repeatedly used to assess long-lasting negative behavioral effects of prolonged isoflurane anesthesia in very young [54,82] and adult [56] mice, reflected by impairments in spatial reversal learning. The latter study in adult mice also reported that melatonin treatment prior to anesthesia prevented such deficits.

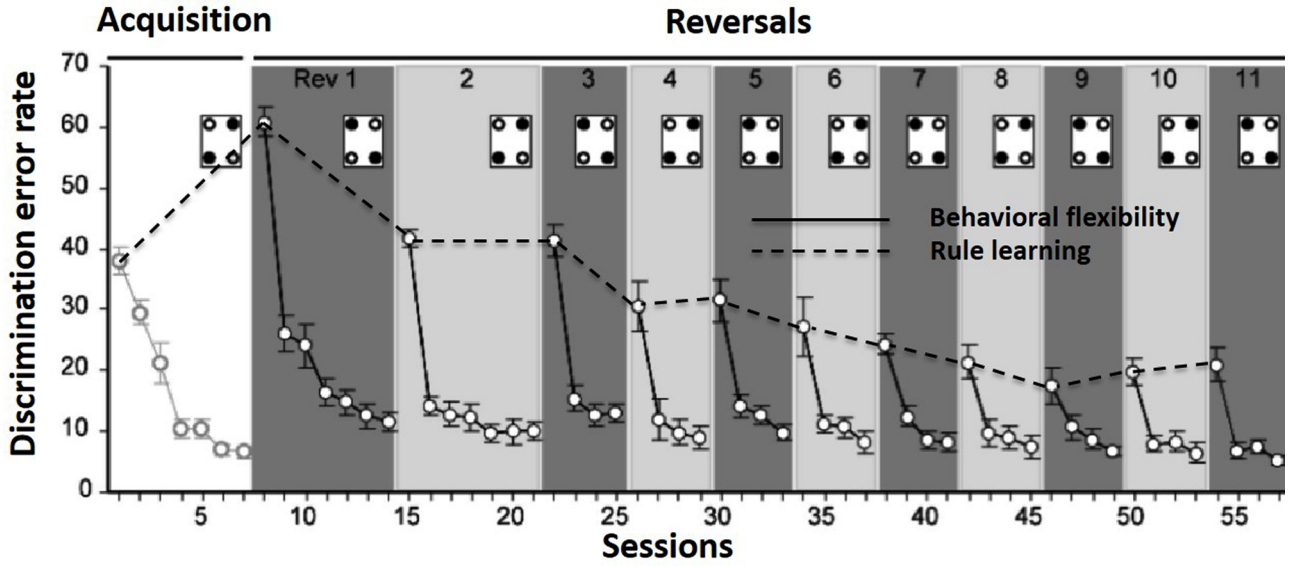


Fig. 5. Behavioral flexibility and rule learning of mice in the IntelliCage. Mice undergo a serial reversal task requiring switching between reward corridors, modelling a human task assessing executive functions (the Brixton spatial anticipation test). Black dots indicate non-rewarded corners, but for getting the next reward, the mouse must visit the opposite diagonal corner. The reversals of the corridor measure behavioral flexibility (speed of adaptation), while the decreasing error rate after multiple reversals indicates rule learning. Modified after Endo et al. [62].

6.2. Effect of anxiogenic and anxiolytic drugs in the IntelliCage

To assess the anxiogenic and anxiolytic effects of drugs, the Vogel conflict paradigm was adapted to the IntelliCage by Safi et al. [83]. C57BL/6 mice were water-deprived and upon drinking from a bottle, they received an air-puff in the corner. The next day, half of the mice received an injection of 5 mg/kg diazepam (i.p.), and the other half received saline at the end of the deprivation period. The first attempt to drink was punished by an air-puff. Compared with control animals, diazepam-treated mice exhibited significantly less anxiety-like behavior, reflected by more visits to the punished corner [83].

It has also been found possible to administer drugs without removing the mice from the IntelliCage for injection. In the experiment presented in Fig. 7, mice were subcutaneously implanted with osmotic minipumps (Alzet) that released the anxiolytic drug alprazolam or saline (controls). Anxiety-like behavior was measured as the latency after receiving an air-puff to the next attempt to visit a corner to drink. Alprazolam-treated mice exhibited lower levels of anxiety-like behavior in the IntelliCage, which was confirmed in the standard elevated plus maze (Fig. 7). Similarly, a shorter latency to re-enter any corner after a visit to the punished corner in female C57BL/6 mice after acute ghrelin administration indicated an anxiolytic-like effect of ghrelin (i.e., an endocrine, hunger stimulating hormone) under acute stressful conditions [84].

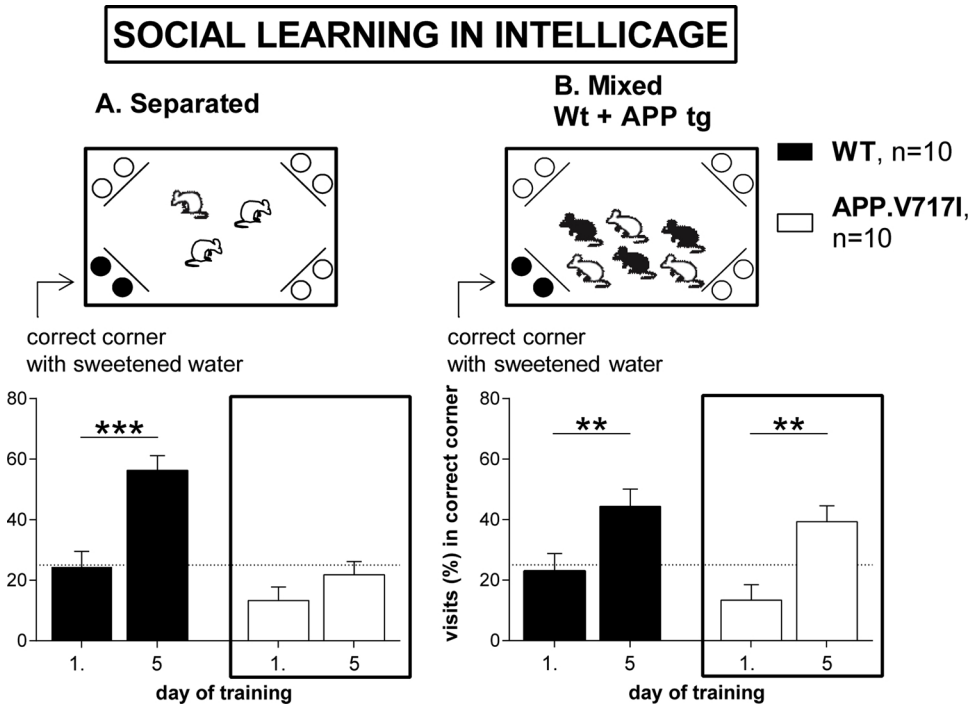


Fig. 6. Group place learning in APP.V717I mice depends on social context. A. After 5 days of learning, APP.V717I mice (white bars) failed to develop a preference for the rewarded corner when housed separately from controls. **B**. When co-housed with control mice, APP.V717I mice developed 40 % preference for the rewarded corner. Wildtype mice (black bars) learned the task independently of housing conditions. The dotted line indicates the random level. $^{**}p < 0.01$, $^{***}p < 0.001$.

6.3. Depression-like behavior

The IntelliCage can also be used to subject mice to controlled stress in a familiar environment to evaluate the impact of drugs on depression-like behavior. Alboni et al. [79] applied various forms of stress in male mice over 14 days by combining a social stressor as described by Branchi et al. [75] and programming stressors in the form of occasional punishment (i.e., air-puffs) when a visit to a drinking corner was made, shortening the time of access when an animal attempted to drink, keeping the doors closed after a nosepoke (which otherwise provided access to drink), and removing shelters and soft bedding material. To test depression-like behavior, the mice received the selective serotonin reuptake inhibitor fluoxetine. The behavioral parameters included the number of corner visits, anhedonia (reflected by saccharin consumption), "wanting" behavior (i.e., the amount of work the animal was willing to perform under a progressive-ratio schedule of reinforcement), and cognitive bias to assess "optimistic attitudes" and "pessimistic attitudes" in individual mice. Interestingly, fluoxetine treatment had opposing behavioral effects during the stressful and stress-free periods [79].

In another experimental protocol in the IntelliCage, 2-week stress exposure in fractalkine receptor knockout mice (i.e., a model of altered responsiveness to chronic stress) did not induce anhedonia compared with wildtype animals [74]. Lower saccharin preference and consumption were observed in mice with sickness behavior syndrome in both the home cage and the IntelliCage [35].

7. Longitudinal studies in the IntelliCage to model human disorders

The ability to assess mouse behavior over a prolonged period of time makes the IntelliCage very useful for developing animal models of progressive diseases. The extended time course of neurodegenerative and neuropsychiatric conditions makes repeated testing of mouse cohorts in conventional apparatus tedious. Examples of such studies that used the IntelliCage system include Balci et al. [85] and Menalled et al. [86], who monitored behavior in a mouse model of Huntington's disease over prolonged periods of time. In follow-up studies, Codita et al. [22] evaluated temporal changes in ArcSwe-APP mice, and Masuda et al. [87] performed long-term phenotyping and validation in genetic mouse models of Alzheimer's disease. The IntelliCage was also recommended as a new technology for preclinical research on animal models of amyotrophic lateral sclerosis (ALS) by the European ALS/Motor Neuron Disease (MND) group in 2009 [88]. Guidelines for testing developmental neurotoxicity have also been suggested to include new methods, such as the IntelliCage system [89]. The potential of such approaches has not yet been fully explored.

Another application that utilizes the IntelliCage for the long-term monitoring of behavior is measurement of the development of drug addiction, which was illustrated by a study that modeled alcohol addiction [72]. Mice in the IntelliCage were first individually characterized for such features as novelty-seeking, anxiety, impulsivity, compulsivity, and the motivation for natural rewards. The same mice were then given extended access to alcohol for 70 days, followed by the evaluation of addiction-like behaviors. The assessment of the level of addiction included the motivation for alcohol on a progressive-ratio schedule of reinforcement, persistent and compulsive alcohol seeking during signaled "no alcohol" periods, responses to punishment, and the intensity of relapse. The data suggested that high levels of anxiety-related traits (i.e., low novelty seeking, low resistance to punishment, a high level of compulsive behaviors and high impulsivity) predicted addiction-like alcohol drinking in mice. In a follow-up study, Stefaniuk et al. [90] used this approach to identify matrix metalloproteinase-9 (MMP-9) as a critical molecule in developing motivation for alcohol seeking behavior.

8. Weaknesses and strengths of the IntelliCage system

8.1. Limitations and concerns

The IntelliCage is a transponder-based system that does not track animals while they traverse the home cage (i.e., outside corners). This raises concerns that only corner-related animal activity is measured in the IntelliCage, such as corner visits, nosepokes, and licks. However, comparative studies of videotracking systems and the IntelliCage indicate a decent correlation between overall activity level and corner visits [85,91,92]. For example, Robinson and Riedel [15] compared the effects of cholinergic, glutamatergic, and dopaminergic system-targeting drugs on activity in the IntelliCage (i.e., corner visits) and in the Phenotyper (Noldus; i.e., total distance moved). They found largely comparable drug profiles, with the exception of one drug in the IntelliCage in which only a trend was found, presumably due to lower spatial resolution. Thus, the number of corner visits appears to be a good proxy for assessing overall activity in individual mice, except for cases in which mice exhibit impairments in the ability to climb into the corners. Building ramps to the tube entries was helpful for overweight mice that had climbing difficulties (H.P. Lipp, unpublished results). Moreover, data that were obtained using the PhenoCube system, which is composed of the IntelliCage and a video tracking system, used also by several groups [86,93–97], measured two mouse models of Huntington's disease (R6/2 and BACHD) over prolonged periods, showing a good correlation between overall activity and corner visits, specifically in older mice [98].

Studies that use auditory stimuli in the IntelliCage are not feasible. Likewise, it has limitations in controlling olfactory stimuli, with the exception of placing simple novel olfactory stimuli in a corner. Unclear is which cues are utilized by mice when they choose a specific corner [99]. Although the IntelliCage provides a social environment, individual interactions between mice cannot be analyzed. This would require spatially fine-grained recordings of movements using RFID tags, which are not yet available, or precise video-tracking of individual mice within a larger group, which is still a challenge for video observation systems.

The IntelliCage cannot generate some types of pronounced stress, such as near-drowning in the Porsolt swim test, severe electric shocks that elicit panic reactions and subsequent immobility (freezing), restraint stress, or pain. The necessity of such stressors and procedures might be justified by specific experimental designs. As shown above, however, the IntelliCage system can generate physiological levels of stress that characterize more common stressful situations.

Another limitation is that IntelliCages are relatively expensive when considered as a single system. However, the simultaneous testing of a comparable number of mice would require the purchase of multiple test systems, in most cases equaling the cost of the IntelliCage system [8].

Although not necessarily a limitation, similar to any other automated system, the IntelliCage generates a large amount of data that require analytical and statistical skills to fully exploit the information. Vannoni et al. [21] described 45 behavioral variables that can be gathered solely during the free adaptation phase. Van Dijk et al. [38] extracted approximately 1500 behavioral readouts in a study that assessed correlations with adult neurogenesis. Although IntelliCage software provides an easy graphic overview of the data, it does not include statistical analyses, which are typically performed using R, SAS, SPSS, Statistica, MatLab, and Python software, among others [100]. Principal component analysis [21,47,49], generalized estimating equations [19,40,51], survival analysis statistics [20], and Bayesian approaches [38] have been applied to analyze IntelliCage datasets. More advanced tools for data analysis, namely R-based statistical software [31] and PyMICE (i.e., an open-source Python library for the analysis of IntelliCage data [101]) have been developed for analyzing output files that are created by various testing schedules in the IntelliCage.

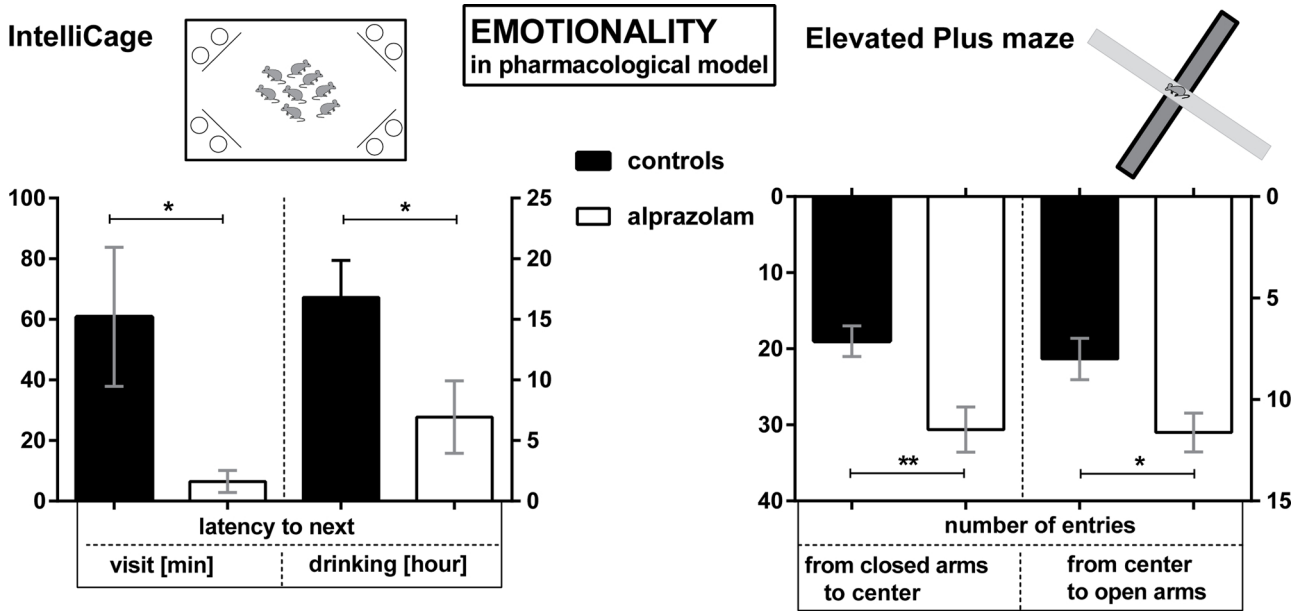


Fig. 7. Decreased anxiety level in mice treated with alprazolam are detected equally by the IntelliCage and elevated plus maze. IntelliCage: A modified Vogel conflict paradigm revealed a strong tendency to return to the corner where an aversive stimulus (air-puff) was delivered in alprazolam-treated mice that attempted to drink water (white bars). Alprazolam-treated mice returned to the same corner after 6 min, whereas control mice took 60 min to return to the same corner. Another attempt to drink occurred after approximately 7 h in alprazolam-treated mice compared with 17 h in controls. **Elevated plus maze**: Alprazolam-treated mice (black bars) left the protected arms more frequently to visit the open center and open arms. $*p < 0.05$, $**p < 0.01$.

8.2. IntelliCage: social sources of variation

A fundamental issue is whether social interactions in the IntelliCage interfere with the proper assessment of individual behavioral phenotypes. Every IntelliCage provides a social micro-environment where animals know each other well, and social hierarchies are established. One concern is that some animals may threaten others by interfering with their programmed learning schedules or generate artificial activity by chasing other group members. Observations of female mice in the IntelliCage, however, do not support this notion. Male mice also find sufficient space and opportunities to perform learning tasks. Generally, strain or treatment differences in the IntelliCage indicate that socially induced variations in spontaneous activity and task solving are of minor importance. Both group and individual behavior can be quantitatively monitored during ongoing studies, and experimenters can scrutinize whether individual mice behave aberrantly. Likewise, testing phases can be prolonged if there is evidence of unexpected behavioral variability without any apparent external cause. If one suspects social effects on learning, then animals can be tested in a given group separately [52]. Finally, new analytical software (e.g., www.xbehavior.com or https://github.com/Neuroinflab/PyMICE_SM/) permit statistical analyses of whether certain mice visit given corners systematically by following other animals, indicating chasing behavior or social affiliation.

The social context can also affect the results, depending on the animal's sex. The use of females in the IntelliCage is recommended unless males must be tested. Notably, the influence of the estrous cycle on the results can be mitigated by long-term, repeated measurements in the IntelliCage. To date, behavioral research using the IntelliCage has been conducted separately in females (47 % of studies), males (37 % of studies), and females and males combined (16 % of studies). Females can live in a group for a long time without overt aggressiveness, whereas the number of males may be reduced because of fighting (14 %) and death (8%) [19,49]. Such adverse effects can be eliminated by prolonged group housing of young males (preferably littermates) before introduction to the IntelliCage. Recently, more studies with males have been performed, both for the overall assessment of behavior and for competitive tests [65,66,102–106]. An innovative approach to generate social stress and measure its effects on depression-like behaviors in the IntelliCage was reported by Branchi et al. [70]. Male mice were kept in cohorts with established social hierarchies in two IntelliCages, and their hedonic behavior (i.e., saccharin preference) was measured. The groups were then mixed and placed again in the IntelliCage. The resulting social stress included lower saccharin consumption (i.e., anhedonia) that was reversed when the original groups were reunited.

8.3. The IntelliCage: ethological features

The IntelliCage creates a stable environment, similar to a home cage, enabling the long-term housing of mice without human intervention. This allows the uninterrupted assessment of unbiased behaviors under no-stress or low-stress conditions over days and weeks, providing individual profiles of activity, including circadian activity patterns, spatial preferences, alternation or perseverance of corner visits (the last one is a useful indicator of cerebral malfunction), and more complex systematic patrolling patterns. Spontaneous behaviors also provide individual activity baselines that are useful for evaluating later spatial or operant conditioning procedures. Moderate changes to the set-up by graphic programming, such as delivering water at limited time slots in specific corners, permit assessment of the sensation of time based on the activity and visits that precede accessibility to the corner (i.e., a proxy for episodic memory).

Another feature of the experimental environment of the IntelliCage that creates preferable living conditions is the social context. Animals of social species perform only a limited repertoire of prosocial behaviors when housed alone [107]. Moreover, long-term social isolation is a classic psychological stressor for rodents, entailing a plethora of uncontrollable effects, including immune disorders [108,109], depression [47,110,111], sleep disorders [112], the accumulation of hippocampal stem cells, but not neurons [113], myelination in the prefrontal cortex [114], and long-lasting epigenetic modifications in the midbrain [6], among others. Individually bred mice have been shown to express greater inter-individual variability than bred in a group [107].

Particularly important is that the IntelliCage system responds to the need to standardize experimental conditions. The influence of contact

with humans, the type of illumination, the size of the apparatus, and social isolation are eliminated, which increases the probability of setting up similar conditions in different laboratories and subsequent replications. This is important for replicating the effects of various treatments or genetic differences on unforced behaviors, which are notoriously sensitive to manual procedures [115,116]. To our knowledge, the IntelliCage is the only system that has generated the same strain differences from four different laboratories using rigorously controlled procedures to assess spontaneous activity and spatial learning [50]. Likewise, parallel IntelliCage studies in Japan and Switzerland reported similar results using a more complex behavioral sequencing task that also included rule learning [62]. Thus, the IntelliCage provides a unique opportunity to conduct meta-analyses of IntelliCage data from different laboratories. For these reasons, work is being conducted to establish a larger version of the IntelliCage system for rats. The first two studies that used rats revealed previously undetected aspects of the phenotype of transgenic Huntington's disease [117] and impairments in recognition memory in rats in which γ-aminobutyric acid-B receptors were modulated [118].

8.4. Comparison with other home cage systems

Currently, many tools are available for the automated long-term collection of behavioral data in home cages, albeit only in single-housed animals. Behavioral assessment includes operant conditioning using an in-cage operant wall (Phenomaster, TSE Systems) and video tracking combined with software that allows limited operant conditioning by directing mice to feeding sites (Phenotyper, Noldus). These systems can also be combined to assess food and water intake and can be placed in metabolic cages (Phenomaster). However, applying automatic monitoring to test more complex behaviors, such as learning and social behaviors, remains technically difficult in these apparatus, and the IntelliCage remains the most flexible system [15,57]. Home cage systems with single animals also present ethical problems when the animal must be tested over prolonged periods of time. Complete isolation for long periods in social species is a practice that is forbidden by European Union ethical standards, unless justified by specific experimental requirements (Directive 2010/63/EU; see also below).

9. IntelliCage: intelligent strategies are required

Automated behavioral testing in the home cage has been criticized, not incorrectly, because it appears to obviate the necessity for experimental thinking, reducing the engagement of behavioral scientists to filling the systems with mice and data mining the output [116]. However, the IntelliCage requires a clear formulation of problems, hypotheses, expected results, and anticipated statistics, combined with knowledge of what a mouse can do. It makes little sense to design complex learning schedules to assess genetic or treatment effects when control mice fail to solve the tasks. On the other hand, spatial learning to find a corner in a home cage that provides a reward is no great challenge, even for a mentally disabled mouse, whereas reversal paradigms usually reveal deficits rapidly. Keeping this in mind, two strategies can be devised, which are not mutually exclusive.

9.1. Strategies for experiment planning: basic protocol

The first strategy consists of automated screening, with a maximum of 2–3 weeks per study, to detect differences in spontaneous activity [21] and simple spatial and operant learning. The goal is primarily to detect behavioral signs of cerebral dysfunction. Obvious candidates for this approach are toxicological models and the enormous number of genetically modified mice. Upon detecting a behavioral difference, this effect should be verified in standard tests to reveal possible emotional, social, cognitive, and pathological mechanisms that may underlie the debilitation. Depending on the outcome, further analyses may use conventional behavioral tests or an IntelliCage that is specifically programmed to reveal deficits in more complex domains, such as impulsivity, behavioral flexibility, or memory impairments. This approach is feasible for the majority of laboratories that possess both the IntelliCage and a standard battery of conventional tests.

In many cases, genetically modified mice that were not generated for neurobiological studies revealed interesting cognitive phenotypes in the IntelliCage. For example, in the field of immunology, such mouse models may provide ideal targets for cognitive research, see the work of Too et al. [27–30,36,119].

9.2. Strategies for experiment planning: advanced protocols

The second strategy is to use the IntelliCage to tackle neurobehavioral problems that cannot be solved by conventional methodologies. This strategy can be employed by laboratories that are experienced with and rely heavily on the IntelliCage system for behavioral analysis. One example is to emotionally profile individual mice and analyze traits that predict the degree of alcohol addiction [72]. Similarly, Alboni et al. [79] manipulated various physiological stress conditions in the IntelliCage to analyze the effects of fluoxetine on depression-like behavior. The above studies would have exceeded the capacity of most behavioral laboratories.

Home cage systems	Advantages	Disadvantages
Phenomaster	in-cage operant wall	single-housed mouse, lack of video tracking
Phenotyper	video tracking, operant conditioning, possible to combined with metabolic cages	single-housed mouse
IntelliCage	social housing, testing of complex behaviors	lack of video tracking

Finally, a recent study by Van Dijk et al. [38] robustly demonstrated the capabilities of the IntelliCage system. They used 106 female C57/BL6 and DBA/2 mice of two different age groups (9 *vs*. 17 weeks), characterized by a peak of adult neurogenesis at 9 weeks and a decline of neurogenesis at 17 weeks [120]. Moreover, the strains differed in their level of adult hippocampal neurogenesis. The mice were tested in the IntelliCage for 30 days with free exploration for the first 7 days, the evaluation of impulsivity (i.e., the ability to withhold a nosepoke during a waiting period) for 17 days (including training), and the

assessment of anxiety by measuring the latency to re-enter a corner where drinking attempt elicited an air-puff. Control mice were kept for the same period of time in the IntelliCage without any testing. The statistical analysis extracted 1457 behavioral readouts. After eliminating inter-correlated variables, the data were checked for individual correlations with the number of newly generated neurons. Surprisingly, the only solid correlation was found in the older mice. Animals with the highest levels of adult neurogenesis exhibited minimal exploratory-like behaviors, which were observed mainly during the first day in the IntelliCage. Although this result indicates an unusual relationship between adult neurogenesis and the response to novelty, it also indicates that many previous studies that analyzed behavioral correlates of adult neurogenesis using standard tests did so in environments that were essentially novel to the animals. Conventional approaches would unlikely have the capability to detect this relationship as well as the IntelliCage.

10. Overall conclusions

Altogether, we believe that the IntelliCage system deserves more attention. Its flexibility in behavioral evaluation is exemplary, covering both simple behavioral screening and more sophisticated cognitive and emotional tests. It avoids many of the pitfalls that are inherent to standard mouse testing, such as handling stress by human experimenters, the lack of standardized environments, procedures and data structures, and limitations with regard to time and manpower to obtain data from sufficiently large sample sizes for solid conclusions to be drawn. Mice undoubtedly like this environment, and maybe scientists can follow.

Declaration of Competing Interest

Prof. Hans-Peter Lipp developed the idea of the IntelliCage based on his original experiments. Prof. Lipp owns shares in NewBehavior Company. The system was initially marketed by NewBehavior GmbH (Zürich, Switzerland), which is now fully owned by TSE-Systems International (Frankfurt, Germany). Prof. Lipp owns a consulting company (Neurospex GmbH) that provides advice for IntelliCage users and also designs other neurobehavioral projects and is co-owner of XBehavior GmbH, a company that provides automated R-based analyses of IntelliCage and GPS tracking data and extended programming capacities for IntelliCage users.

Acknowledgements

Funding was provided by a Swiss-Polish grant from Switzerland through the Swiss Contribution to the enlarged European Union (PSPB-210/2010 to E.K. and H.P.L.) and the ANIMOD project within the Team Tech Core Facility Plus program of the Foundation for Polish Science, co-financed by the European Union under the European Regional Development Fund to W.K. EK & LK acknowledge support from the BRAINCITY MAB/2018/10. The BRAINCITY project is performed within the International Research Agendas program of the Foundation for Polish Science, co-financed by the European Union under the European Regional Development Fund.

References

[1] S. Ramboz, R. Oosting, D.A. Amara, H.F. Kung, P. Blier, M. Mendelsohn, J.J. Mann, D. Brunner, R. Hen, Serotonin receptor 1A knockout: an animal model of anxiety-related disorder, Proc. Natl. Acad. Sci. U. S. A. 95 (24) (1998) 14476–14481.

[2] L.K. Heisler, H.M. Chu, T.J. Brennan, J.A. Danao, P. Bajwa, L.H. Parsons, L.H. Tecott, Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice, Proc. Natl. Acad. Sci. U. S. A. 95 (25) (1998) 15049–15054.

[3] F. Saudou, D.A. Amara, A. Dierich, M. LeMeur, S. Ramboz, L. Segu, M.C. Buhot, R. Hen, Enhanced aggressive behavior in mice lacking 5-HT1B receptor, Science 265 (5180) (1994) 1875–1878.

[4] H. Bueler, M. Fischer, Y. Lang, H. Bluethmann, H.P. Lipp, S.J. DeArmond, S.B. Prusiner, M. Aguet, C. Weissmann, Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein, Nature 356 (6370) (1992) 577–582.

[5] M.E. Bach, R.D. Hawkins, M. Osman, E.R. Kandel, M. Mayford, Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency, Cell 81 (6) (1995) 905–915.

[6] D. Siuda, Z. Wu, Y. Chen, L. Guo, M. Linke, U. Zechner, N. Xia, G. Reifenberg, H. Kleinert, U. Forstermann, H. Li, Social isolation-induced epigenetic changes in midbrain of adult mice, J. Physiol. Pharmacol. 65 (2) (2014) 247–255.

[7] H.-P. Lipp, O. Litvin, M. Galsworthy, D.L. Vyssotsky, A.L. Vyssotsky, P. Zinn, A.E. Rau, F. Neuhäusser-Wespy, H. Würbel, R. Nitsch, D.P. Wolfer, Automated behavioral analysis of mice using INTELLICAGE: inter-laboratory comparisons and validation with exploratory behavior and spatial learning, in: L.P.P.J. Noldus, F. Grieco, L.W.S. Loijens, P.H. Zimmermann (Eds.), Proceedings of Measuring Behavior 2005 Noldus, Wageningen, 2005, pp. 66–69.

[8] H.-P. Lipp, High-throughput and automated behavioural screening of normal and genetically modified mice, Business Briefing: Future Drug Discovery 5 (2005).

[9] G. Dell'Omo, L. Ricceri, D.P. Wolfer, I.I. Poletaeva, H. Lipp, Temporal and spatial adaptation to food restriction in mice under naturalistic conditions, Behav. Brain Res. 115 (1) (2000) 1–8.

[10] A.L. Vyssotski, G. Dell'Omo, I.I. Poletaeva, D.L. Vyssotsk, L. Minichiello, R. Klein, D.P. Wolfer, H.P. Lipp, Long-term monitoring of hippocampus-dependent behavior in naturalistic settings: mutant mice lacking neurotrophin receptor TrkB in the forebrain show spatial learning but impaired behavioral flexibility, Hippocampus 12 (1) (2002) 27–38.

[11] A. Abbott, Behavioural genetics: a question of survival, Nature 449 (7162) (2007) 532–534.

[12] N. Kulesskaya, V. Voikar, M. Peltola, G.G. Yegutkin, M. Salmi, S. Jalkanen, H. Rauvala, CD73 is a major regulator of adenosinergic signalling in mouse brain, PLoS One 8 (6) (2013) e66896.

[13] S.W. Weyer, M. Klevanski, A. Delekate, V. Voikar, D. Aydin, M. Hick, M. Filippov, N. Drost, K.L. Schaller, M. Saar, M.A. Vogt, P. Gass, A. Samanta, A. Jaschke, M. Korte, D.P. Wolfer, J.H. Caldwell, U.C. Muller, APP and APLP2 are essential at PNS and CNS synapses for transmission, spatial learning and LTP, EMBO J. 30 (11) (2011) 2266–2280.

[14] P. Jedynak, P. Jaholkowski, G. Wozniak, C. Sandi, L. Kaczmarek, R.K. Filipkowski, Lack of cyclin D2 impairing adult brain neurogenesis alters hippocampal-dependent behavioral tasks without reducing learning ability, Behav. Brain Res. 227 (1) (2012) 159–166.

[15] L. Robinson, G. Riedel, Comparison of automated home-cage monitoring systems: emphasis on feeding behaviour, activity and spatial learning following pharmacological interventions, J. Neurosci. Methods 234 (2014) 13–25.

[16] O. Rudenko, V. Tkach, V. Berezin, E. Bock, Detection of early behavioral markers of Huntington's disease in R6/2 mice employing an automated social home cage, Behav. Brain Res. 203 (2) (2009) 188–199.

[17] J. Viosca, A.J. Schuhmacher, C. Guerra, A. Barco, Germline expression of H-RasG12V causes neurological deficits associated to Costello syndrome, Genes Brain Behav. 8 (1) (2009) 60–71.

[18] A. Kiryk, T. Aida, K. Tanaka, P. Banerjee, G.M. Wilczynski, K. Meyza, E. Knapska, R.K. Filipkowski, L. Kaczmarek, W. Danysz, Behavioral characterization of GLT1 (+/-) mice as a model of mild glutamatergic hyperfunction, Neurotox. Res. 13 (1) (2008) 19–30.

[19] N. Karlsson, M. Kalm, M.K.L. Nilsson, C. Mallard, T. Björk-Eriksson, K. Blomgren, Learning and activity after irradiation of the young mouse brain analyzed in adulthood using unbiased monitoring in a home cage environment, Radiat. Res. 175 (3) (2011) 336–346.

[20] M.J. Galsworthy, I. Amrein, P.A. Kuptsov, I.I. Poletaeva, P. Zinn, A. Rau, A. Vyssotski, H.P. Lipp, A comparison of wild-caught wood mice and bank voles in the Intellicage: assessing exploration, daily activity patterns and place learning paradigms, Behav. Brain Res. 157 (2) (2005) 211–217.

[21] E. Vannoni, V. Voikar, G. Colacicco, M.A. Sánchez, H.-P. Lipp, D.P. Wolfer, Spontaneous behavior in the social homecage discriminates strains, lesions and mutations in mice, J. Neurosci. Methods 234 (2014) 26–37.

[22] A. Codita, A. Gumucio, L. Lannfelt, P. Gellerfors, B. Winblad, A.H. Mohammed, L.N.G. Nilsson, Impaired behavior of female tg-ArcSwe APP mice in the IntelliCage: a longitudinal study, Behav. Brain Res. 215 (1) (2010) 83–94.

[23] V. Voikar, N. Kulesskaya, T. Laakso, J. Lauren, S.M. Strittmatter, M.S. Airaksinen, LRRTM1-deficient mice show a rare phenotype of avoiding small enclosures–a tentative mouse model for claustrophobia-like behaviour, Behav. Brain Res. 238 (2013) 69–78.

[24] M. Heidari, D.M. Johnstone, B. Bassett, R.M. Graham, A.C. Chua, M.J. House, J.F. Collingwood, C. Bettencourt, H. Houlden, M. Ryten, J.K. Olynyk, D. Trinder, E.A. Milward, Brain iron accumulation affects myelin-related molecular systems implicated in a rare neurogenetic disease family with neuropsychiatric features, Mol. Psychiatry (2016).

[25] O. Ermakova, L. Piszczek, L. Luciani, F.M. Cavalli, T. Ferreira, D. Farley, S. Rizzo, R.C. Paolicelli, M. Al-Banchaabouchi, C. Nerlov, R. Moriggl, N.M. Luscombe, C. Gross, Sensitized phenotypic screening identifies gene dosage sensitive region on chromosome 11 that predisposes to disease in mice, EMBO Mol. Med. 3 (1) (2011) 50–66.

[26] L.K. Too, K.M. Li, C. Suarna, G.J. Maghzal, R. Stocker, I.S. McGregor, N.H. Hunt, Deletion of TDO2, IDO-1 and IDO-2 differentially affects mouse behavior and cognitive function, Behav. Brain Res. 312 (2016) 102–117.

[27] L.K. Too, H.J. Ball, I.S. McGregor, N.H. Hunt, A novel automated test battery reveals enduring behavioural alterations and cognitive impairments in survivors of murine pneumococcal meningitis, Brain Behav. Immun. 35 (2014) 107–124.
[28] L.K. Too, H.J. Ball, I.S. McGregor, N.H. Hunt, The pro-inflammatory cytokine interferon-gamma is an important driver of neuropathology and behavioural sequelae in experimental pneumococcal meningitis, Brain, Behavior, Immunity 40 (2014) 252–268.
[29] L.K. Too, J.A. McQuillan, H.J. Ball, M. Kanai, T. Nakamura, H. Funakoshi, I.S. McGregor, N.H. Hunt, The kynurenine pathway contributes to long-term neuropsychological changes in experimental pneumococcal meningitis, Behav. Brain Res. 270 (2014) 179–195.
[30] L.K. Too, A.J. Mitchell, B. Yau, H.J. Ball, I.S. McGregor, N.H. Hunt, Interleukin-18 deficiency and its long-term behavioural and cognitive impacts in a murine model of pneumococcal meningitis, Behav. Brain Res. 263 (2014) 176–189.
[31] V. Voikar, S. Krackow, H.-P. Lipp, A. Rau, G. Colacicco, D.P. Wolfer, Automated dissection of permanent effects of hippocampal or prefrontal lesions on performance at spatial, working memory and circadian timing tasks of C57BL/6 mice in IntelliCage, Behav. Brain Res. 352 (2018) 8–22.
[32] S. Muthuraju, S. Pati, M. Rafiqul, J.F. Abdullah, H. Jaafar, IntelliCage provides voluntary exercise and an enriched environment, improving locomotive activity in mice following fluid percussion injury, Basal Ganglia 2 (2012) 143–151.
[33] Y. Kobayashi, Y. Sano, E. Vannoni, H. Goto, H. Suzuki, A. Oba, H. Kawasaki, S. Kanba, H.P. Lipp, N.P. Murphy, D.P. Wolfer, S. Itohara, Genetic dissection of medial habenula-interpeduncular nucleus pathway function in mice, Front. Behav. Neurosci. 7 (2013) 17.
[34] N. Onishchenko, C. Tamm, M. Vahter, T. Hökfelt, J.A. Johnson, D.A. Johnson, S. Ceccatelli, Developmental exposure to methylmercury alters learning and induces depression-like behavior in male mice, Toxicol. Sci. 97 (2) (2007) 428–437.
[35] F. Cathomas, R. Fuertig, H. Sigrist, G.N. Newman, V. Hoop, M. Bizzozzero, A. Mueller, A. Luippold, A. Ceci, B. Hengerer, E. Seifritz, A. Fontana, C.R. Pryce, CD40-TNF activation in mice induces extended sickness behavior syndrome coincident with but not dependent on activation of the kynurenine pathway, Brain Behav. Immun. 50 (2015) 125–140.
[36] L.K. Too, A.J. Mitchell, I.S. McGregor, N.H. Hunt, Antibody-induced neutrophil depletion prior to the onset of pneumococcal meningitis influences long-term neurological complications in mice, Brain Behav. Immun. 56 (2016) 68–83.
[37] H.P. Lipp, High-throughput and automated behavioural screening of normal and genetically modified mice, Drug Discov. Today (2005) 1–5.
[38] R.M. van Dijk, S.E. Lazic, L. Slomianka, D.P. Wolfer, I. Amrein, Large-scale phenotyping links adult hippocampal neurogenesis to the reaction to novelty: large scale phenotyping links neurogenesis to novelty, Hippocampus 26 (5) (2016) 646–657.
[39] S.A. Barnett, Experiments on neophobia in wild and laboratory rats, Br. J. Psychol. 49 (3) (1958) 195–201.
[40] K. Huo, Y. Sun, H. Li, X. Du, X. Wang, N. Karlsson, C. Zhu, K. Blomgren, Lithium reduced neural progenitor apoptosis in the hippocampus and ameliorated functional deficits after irradiation to the immature mouse brain, Mol. Cell. Neurosci. 51 (1-2) (2012) 32–42.
[41] W. Konopka, A. Kiryk, M. Novak, M. Herwerth, J.R. Parkitna, M. Wawrzyniak, A. Kowarsch, P. Michaluk, J. Dzwonek, T. Arnsperger, G. Wilczynski, M. Merkenschlager, F.J. Theis, G. Kohr, L. Kaczmarek, G. Schutz, MicroRNA loss enhances learning and memory in mice, J. Neurosci. 30 (44) (2010) 14835–14842.
[42] D. Ryan, D. Koss, E. Porcu, H. Woodcock, L. Robinson, B. Platt, G. Riedel, Spatial learning impairments in PLB1Triple knock-in Alzheimer mice are task-specific and age-dependent, Cell. Mol. Life Sci. 70 (14) (2013) 2603–2619.
[43] K. Lee, Y. Kobayashi, H. Seo, J.-H. Kwak, A. Masuda, C.-S. Lim, H.-R. Lee, S.J. Kang, P. Park, S.-E. Sim, N. Kogo, H. Kawasaki, B.-K. Kaang, S. Itohara, Involvement of cAMP-guanine nucleotide exchange factor II in hippocampal long-term depression and behavioral flexibility, Mol. Brain 8 (1) (2015).
[44] W.C. Lan, M. Priestley, S.R. Mayoral, L. Tian, M. Shamloo, A.A. Penn, Sex-specific cognitive deficits and regional brain volume loss in mice exposed to chronic, sublethal hypoxia, Pediatr. Res. 70 (1) (2011) 15–20.
[45] P. Jaholkowski, A. Kiryk, P. Jedynak, N.M. Ben Abdallah, E. Knapska, A. Kowalczyk, A. Piechal, K. Blecharz-Klin, I. Figiel, V. Lioudyno, E. Widy-Tyszkiewicz, G.M. Wilczynski, H.P. Lipp, L. Kaczmarek, R.K. Filipkowski, New hippocampal neurons are not obligatory for memory formation; cyclin D2 knockout mice with no adult brain neurogenesis show learning, Learn. Mem. 16 (7) (2009) 439–451.
[46] A. Barlind, N. Karlsson, T. Björk-Eriksson, J. Isgaard, K. Blomgren, Decreased cytogenesis in the granule cell layer of the hippocampus and impaired place learning after irradiation of the young mouse brain evaluated using the IntelliCage platform, Exp. Brain Res. 201 (4) (2010) 781–787.
[47] A. Berry, I. Amrein, S. Nötzli, S.E. Lazic, V. Bellisario, M. Giorgio, P.G. Pelicci, E. Alleva, H.-P. Lipp, F. Cirulli, Sustained hippocampal neurogenesis in females is amplified in P66Shc−/− mice: an animal model of healthy aging, Hippocampus 22 (12) (2012) 2249–2259.
[48] M. Faizi, P.L. Bader, C. Tun, A. Encarnacion, A. Kleschevnikov, P. Belichenko, N. Saw, M. Priestley, R.W. Tsien, W.C. Mobley, M. Shamloo, Comprehensive behavioral phenotyping of Ts65Dn mouse model of Down Syndrome: activation of β1-adrenergic receptor by xamoterol as a potential cognitive enhancer, Neurobiol. Dis. 43 (2) (2011) 397–413.
[49] M. Kalm, N. Karlsson, M.K.L. Nilsson, K. Blomgren, Loss of hippocampal neurogenesis, increased novelty-induced activity, decreased home cage activity, and impaired reversal learning one year after irradiation of the young mouse brain, Exp. Neurol. 247 (2013) 402–409.
[50] S. Krackow, E. Vannoni, A. Codita, A.H. Mohammed, F. Cirulli, I. Branchi, E. Alleva, A. Reichelt, A. Willuweit, V. Voikar, G. Colacicco, D.P. Wolfer, J.-U.F. Buschmann, K. Safi, H.-P. Lipp, Consistent behavioral phenotype differences between inbred mouse strains in the IntelliCage, Genes, Brain Behav. 9 (7) (2010) 722–731.
[51] K. Roughton, M. Kalm, K. Blomgren, Sex-dependent differences in behavior and hippocampal neurogenesis after irradiation to the young mouse brain: gender differences after IR to the rodent brain, Eur. J. Neurosci. 36 (6) (2012) 2763–2772.
[52] A. Kiryk, G. Mochol, R.K. Filipkowski, M. Wawrzyniak, V. Lioudyno, E. Knapska, T. Gorkiewicz, M. Balcerzyk, S. Leski, F.V. Leuven, H.P. Lipp, D.K. Wojcik, L. Kaczmarek, Cognitive abilities of Alzheimer's disease transgenic mice are modulated by social context and circadian rhythm, Curr. Alzheimer Res. 8 (8) (2011) 883–892.
[53] E. Knapska, G. Walasek, E. Nikolaev, F. Neuhausser-Wespy, H.P. Lipp, L. Kaczmarek, T. Werka, Differential involvement of the central amygdala in appetitive versus aversive learning, Learn. Mem. 13 (2) (2006) 192–200.
[54] C. Zhu, J. Gao, N. Karlsson, Q. Li, Y. Zhang, Z. Huang, H. Li, H.G. Kuhn, K. Blomgren, Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents, J. Cerebral Blood Flow & Metabol. 30 (5) (2010) 1017–1030.
[55] A. Codita, A.H. Mohammed, A. Willuweit, A. Reichelt, E. Alleva, I. Branchi, F. Cirulli, G. Colacicco, V. Voikar, D.P. Wolfer, F.J. Buschmann, H.P. Lipp, E. Vannoni, S. Krackow, Effects of spatial and cognitive enrichment on activity pattern and learning performance in three strains of mice in the IntelliMaze, Behav Genet 42 (3) (2012) 449–460.
[56] T. Xia, Y. Cui, S. Chu, J. Song, Y. Qian, Z. Ma, X. Gu, Melatonin pretreatment prevents isoflurane-induced cognitive dysfunction by modulating sleep-wake rhythm in mice, Brain Res (2015).
[57] G.J. Ramakers, D. Wolfer, G. Rosenberger, K. Kuchenbecker, H.J. Kreienkamp, J. Prange-Kiel, G. Rune, K. Richter, K. Langnaese, S. Masneuf, M.R. Bosl, K.D. Fischer, H.J. Krugers, H.P. Lipp, E. van Galen, K. Kutsche, Dysregulation of Rho GTPases in the alphaPix/Arhgef6 mouse model of X-linked intellectual disability is paralleled by impaired structural and synaptic plasticity and cognitive deficits, Hum Mol Genet 21 (2) (2012) 268–286.
[58] A.O. Mechan, A. Wyss, H. Rieger, M.H. Mohajeri, A comparison of learning and memory characteristics of young and middle-aged wild-type mice in the IntelliCage, Journal of Neuroscience Methods 180 (1) (2009) 43–51.
[59] A.M. Osman, K. Zhou, C. Zhu, K. Blomgren, Transplantation of enteric neural Stem/Progenitor cells into the irradiated young mouse Hippocampus, Cell Transplantation 23 (12) (2014) 1657–1671.
[60] M. Kalm, U. Andreasson, T. Bjork-Eriksson, H. Zetterberg, M. Pekny, K. Blennow, M. Pekna, K. Blomgren, C3 deficiency ameliorates the negative effects of irradiation of the young brain on hippocampal development and learning, Oncotarget 7 (15) (2016) 19382–19394.
[61] K. Gapp, S. Soldado-Magraner, M. Alvarez-Sanchez, J. Bohacek, G. Vernaz, H. Shu, T.B. Franklin, D. Wolfer, I.M. Mansuy, Early life stress in fathers improves behavioural flexibility in their offspring, Nat Commun 5 (2014) 5466.
[62] T. Endo, F. Maekawa, V. Võikar, A. Haijima, Y. Uemura, Y. Zhang, W. Miyazaki, S. Suyama, K. Shimazaki, D.P. Wolfer, T. Yada, C. Tohyama, H.-P. Lipp, M. Kakeyama, Automated test of behavioral flexibility in mice using a behavioral sequencing task in IntelliCage, Behav. Brain Res. 221 (1) (2011) 172–181.
[63] J.A. Vordenberg, J.J. Barrett, N.A. Doninger, C.P. Contardo, K.A. Ozoude, Application of the Brixton spatial anticipation test in stroke: ecological validity and performance characteristics, Clin Neuropsychol 28 (2) (2014) 300–316.
[64] M. Smutek, M. Turbasa, M. Sikora, M. Piechota, J. Zajdel, R. Przewlocki, J.R. Parkitna, A model of alcohol drinking under an intermittent access schedule using group-housed mice, PLoS ONE 9 (5) (2014) e96787.
[65] S. Benner, T. Endo, N. Endo, M. Kakeyama, C. Tohyama, Early deprivation induces competitive subordinance in C57BL/6 male mice, Physiol. Behav. 137 (2014) 42–52.
[66] T. Endo, M. Kakeyama, Y. Uemura, A. Haijima, H. Okuno, H. Bito, C. Tohyama, Executive function deficits and social-behavioral abnormality in mice exposed to a low dose of dioxin in utero and via lactation, PLoS ONE 7 (12) (2012) e50741.
[67] K. Ishii, K.-i. Kubo, T. Endo, K. Yoshida, S. Benner, Y. Ito, H. Aizawa, M. Aramaki, A. Yamanaka, K. Tanaka, N. Takata, K.F. Tanaka, M. Mimura, C. Tohyama, M. Kakeyama, K. Nakajima, Neuronal heterotopias affect the activities of distant brain areas and lead to behavioral deficits, J. Neurosci. 35 (36) (2015) 12432–12445.
[68] P. Carlier, M. Jamon, Observational learning in C57BL/6j mice, Behav Brain Res 174 (1) (2006) 125–131.
[69] A. Nowak, T. Werka, E. Knapska, Social modulation in extinction of aversive memories, Behav. Brain Res. 238 (2013) 200–205.
[70] I. Branchi, I. D'Andrea, F. Cirulli, H.-P. Lipp, E. Alleva, Shaping brain development: mouse communal nesting blunts adult neuroendocrine and behavioral response to social stress and modifies chronic antidepressant treatment outcome, Psychoneuroendocrinology 35 (5) (2010) 743–751.
[71] M. Patrikainen, P. Pan, N. Kulesskaya, V. Voikar, S. Parkkila, The role of carbonic anhydrase VI in bitter taste perception: evidence from the Car6(-)/(-) mouse model, J Biomed Sci 21 (2014) 82.
[72] K. Radwanska, L. Kaczmarek, Characterization of an alcohol addiction-prone phenotype in mice, Addict Biol 17 (3) (2012) 601–612.
[73] E. Knapska, V. Lioudyno, A. Kiryk, M. Mikosz, T. Gorkiewicz, P. Michaluk, M. Gawlak, M. Chaturvedi, G. Mochol, M. Balcerzyk, D.K. Wojcik, G.M. Wilczynski, L. Kaczmarek, Reward learning requires activity of matrix Metalloproteinase-9 in the Central Amygdala, Journal of Neuroscience 33 (36) (2013) 14591–14600.

[74] G. Milior, C. Lecours, L. Samson, K. Bisht, S. Poggini, F. Pagani, C. Deflorio, C. Lauro, S. Alboni, C. Limatola, I. Branchi, M.E. Tremblay, L. Maggi, Fractalkine receptor deficiency impairs microglial and neuronal responsiveness to chronic stress, Brain Behav Immun (2015).
[75] I. Branchi, S. Santarelli, S. Capoccia, S. Poggini, I. D'Andrea, F. Cirulli, E. Alleva, Antidepressant treatment outcome depends on the quality of the living environment: a pre-clinical investigation in mice, PLoS One 8 (4) (2013) e62226.
[76] N. Kulesskaya, N.N. Karpova, L. Ma, L. Tian, V. Voikar, Mixed housing with DBA/2 mice induces stress in C57BL/6 mice: implications for interventions based on social enrichment, Frontiers in Behav. Neurosci. 8 (2014).
[77] J.R. Parkitna, M. Sikora, S. Golda, K. Golembiowska, B. Bystrowska, D. Engblom, A. Bilbao, R. Przewlocki, Novelty-seeking behaviors and the escalation of alcohol drinking after abstinence in mice are controlled by metabotropic glutamate receptor 5 on neurons expressing dopamine d1 receptors, Biol Psychiatry 73 (3) (2013) 263–270.
[78] S. Marwari, G.S. Dawe, Effects of haloperidol on cognitive function and behavioural flexibility in the IntelliCage social home cage environment, Behav. Brain Res. 371 (2019) 111976.
[79] S. Alboni, R.M. van Dijk, S. Poggini, G. Milior, M. Perrotta, T. Drenth, N. Brunello, D.P. Wolfer, C. Limatola, I. Amrein, F. Cirulli, L. Maggi, I. Branchi, Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment, Mol Psychiatry (2015).
[80] D.C. Ajonijebu, O. Abboussi, M.V. Mabandla, W.M.U. Daniels, Cocaine-induced inheritable epigenetic marks may be altered by changing early postnatal fostering, NeuroReport 30 (17) (2019) 1157–1165.
[81] E. Vazquez, A. Barranco, M. Ramirez, A. Gruart, J.M. Delgado-Garcia, E. Martinez-Lara, S. Blanco, M.J. Martin, E. Castanys, R. Buck, P. Prieto, R. Rueda, Effects of a human milk oligosaccharide, 2'-fucosyllactose, on hippocampal long-term potentiation and learning capabilities in rodents, J Nutr Biochem 26 (5) (2015) 455–465.
[82] Y. Yang, X. Chen, H. Min, S. Song, J. Zhang, S. Fan, L. Yi, H. Wang, X. Gu, Z. Ma, Q. Gao, Persistent mitoK activation is involved in the isoflurane-induced cytotoxicity, Mol Neurobiol (2016).
[83] K. Safi, F. Neuhäusser-Wespy, H. Welzl, H.-P. Lipp, Mouse Anxiety Models and an Example of an Experimental Setup Using Unconditioned Avoidance in an Automated System - Intellicage, (2006) 15.
[84] M. Jensen, C. Ratner, O. Rudenko, S.H. Christiansen, L.J. Skov, C. Hundahl, D.P. Woldbye, B. Holst, Anxiolytic-like effects of increased ghrelin receptor signaling in the Amygdala, Int. J. Neuropsychopharmacol (2015).
[85] F. Balci, S. Oakeshott, J.L. Shamy, B.F. El-Khodor, I. Filippov, R. Mushlin, R. Port, D. Connor, A. Paintdakhi, L. Menalled, S. Ramboz, D. Howland, S. Kwak, D. Brunner, High-throughput automated phenotyping of two genetic mouse models of Huntington's disease, PLoS Curr 5 (2013).
[86] L.B. Menalled, A.E. Kudwa, S. Miller, J. Fitzpatrick, J. Watson-Johnson, N. Keating, M. Ruiz, R. Mushlin, W. Alosio, K. McConnell, D. Connor, C. Murphy, S. Oakeshott, M. Kwan, J. Beltran, A. Ghavami, D. Brunner, L.C. Park, S. Ramboz, D. Howland, Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175, PLoS ONE 7 (12) (2012) e49838.
[87] A. Masuda, Y. Kobayashi, N. Kogo, T. Saito, T.C. Saido, S. Itohara, Cognitive deficits in single App knock-in mouse models, Neurobiol. Learning and Memory 135 (2016) 73–82.
[88] A.C. Ludolph, C. Bendotti, E. Blaugrund, A. Chio, L. Greensmith, J.P. Loeffler, R. Mead, H.G. Niessen, S. Petri, P.F. Pradat, W. Robberecht, M. Ruegg, B. Schwalenstocker, D. Stiller, L. van den Berg, F. Vieira, S. von Horsten, Guidelines for preclinical animal research in ALS/MND: a consensus meeting, Amyotroph Lateral Scler 11 (1-2) (2010) 38–45.
[89] C. Tohyama, Dvelopmental neurotoxicity guidelines: problems and perspectievs, J. Toxicol.Sci 41 (Special issue) (2016) 69–79.
[90] M. Stefaniuk, A. Beroun, T. Lebitko, O. Markina, S. Leski, K. Meyza, A. Grzywacz, J. Samochowiec, A. Samochowiec, K. Radwanska, L. Kaczmarek, Matrix Metalloproteinase-9 and synaptic plasticity in the Central Amygdala in control of alcohol-seeking behavior, Biological Psychiatry 81 (11) (2017) 907–917.
[91] L.B. Menalled, A.E. Kudwa, S. Oakeshott, A. Farrar, N. Paterson, I. Filippov, S. Miller, M. Kwan, M. Olsen, J. Beltran, J. Torello, J. Fitzpatrick, R. Mushlin, K. Cox, K. McConnell, M. Mazzella, D. He, G.F. Osborne, R. Al-Nackkash, G.P. Bates, P. Tuunanen, K. Lehtimaki, D. Brunner, A. Ghavami, S. Ramboz, L. Park, D. Macdonald, I. Munoz-Sanjuan, D. Howland, Genetic deletion of transglutaminase 2 does not rescue the phenotypic deficits observed in R6/2 and zQ175 mouse models of Huntington's disease, PLoS ONE 9 (6) (2014) e99520.
[92] S. Oakeshott, F. Balci, I. Filippov, C. Murphy, R. Port, D. Connor, A. Paintdakhi, J. LeSauter, L. Menalled, S. Ramboz, S. Kwak, D. Howland, R. Silver, D. Brunner, Circadian abnormalities in motor activity in a BAC transgenic mouse model of Huntington's disease, PLoS Currents 3 (2011) RRN1225.
[93] V. Alexandrov, D. Brunner, T. Hanania, E. Leahy, Reprint of: highthroughtput analysis of behavior for drug discovery, European J. Pharmacol. 753 (2015) 127–134.
[94] V. Alexandrov, D. Brunner, L.B. Menalled, A. Kudwa, J. Watson-Johnson, M. Mazzella, I. Russell, M.C. Ruiz, J. Torello, E. Sabath, A. Sanchez, M. Gomez, I. Filipov, K. Cox, M. Kwan, A. Ghavami, S. Ramboz, B. Lager, V.C. Wheeler, J. Aaronson, J. Rosinski, J.F. Gusella, M.E. MacDonald, D. Howland, S. Kwak, Large-scale phenome analysis defines a behavioral signature for Huntington's disease genotype in mice, Nature Biotechnol. 34 (8) (2016) 838–844.
[95] A. Benraiss, S. Wang, S. Herrlinger, X. Li, D. Chandler-Militello, J. Mauceri, H.B. Burm, M. Toner, M. Osipovitch, Q. Jim Xu, F. Ding, F. Wang, N. Kang, J. Kang, P.C. Curtin, D. Brunner, M.S. Windrem, I. Munoz-Sanjuan, M. Nedergaard, S.A. Goldman, Human glia can both induce and rescue aspects of disease phenotype in Huntington disease, Nature Commun. 7 (1) (2016).
[96] S. Oakeshott, A. Farrar, R. Port, J. Cummins-Sutphen, J. Berger, J. Watson-Johnson, S. Ramboz, D. Howland, D. Brunner, Deficits in a simple visual Go/No-go discrimination task in two mouse models of Huntington's disease, PLoS Currents (2013).
[97] S. Oakeshott, R. Port, J. Cummins-Sutphen, J. Berger, J. Watson-Johnson, S. Ramboz, N. Paterson, S. Kwak, D. Howland, D. Brunner, A mixed fixed ratio/progressive ratio procedure reveals an apathy phenotype in the BAC HD and the z_Q175 KI mouse models of Huntington's disease, PLoS Currents (2012).
[98] I. Branchi, S. Santarelli, I. D'Andrea, E. Alleva, Not all stressors are equal: early social enrichment favors resilience to social but not physical stress in male mice, Hormones and Behavior 63 (3) (2013) 503–509.
[99] D. Wahlsten, Mouse Behavioral Testing: How to Use Mice in Behavioral Neuroscience, Elsevier Academic Press, 2010.
[100] A. Puscian, S. Leski, T. Gorkiewicz, K. Meyza, H.P. Lipp, E. Knapska, A novel automated behavioral test battery assessing cognitive rigidity in two genetic mouse models of autism, Front Behav Neurosci 8 (2014) 140.
[101] J.M. Dzik, A. Puścian, Z. Mijakowska, K. Radwanska, S. Łęski, PyMICE: A Python library for analysis of IntelliCage data, Behavior Res. Methods 50 (2) (2018) 804–815.
[102] T.M. Kato, M. Kubota-Sakashita, N. Fujimori-Tonou, F. Saitow, S. Fuke, A. Masuda, S. Itohara, H. Suzuki, T. Kato, Ant1 mutant mice bridge the mitochondrial and serotonergic dysfunctions in bipolar disorder, Molecular Psychiatry 23 (10) (2018) 2039–2049.
[103] S. Poggini, M.T. Golia, S. Alboni, G. Milior, L.P. Sciarria, A. Viglione, G. Matte Bon, N. Brunello, S. Puglisi-Allegra, C. Limatola, L. Maggi, I. Branchi, Combined Fluoxetine and Metformin Treatment Potentiates Antidepressant Efficacy Increasing IGF2 Expression in the Dorsal Hippocampus, Neural Plasticity 2019 (2019) 1–12.
[104] Y. Sato, N. Shinjyo, M. Sato, M.K.L. Nilsson, K. Osato, C. Zhu, M. Pekna, H.G. Kuhn, K. Blomgren, Grafting neural stem and progenitor cells into the Hippocampus of juvenile, irradiated mice normalizes behavior deficits, Frontiers in Neurology 9 (2018).
[105] U. Wilhelmsson, M. Kalm, M. Pekna, M. Pekny, Nestin null mice show improved reversal place learning, Neurochem. Res. (2019).
[106] U. Wilhelmsson, A. Pozo-Rodrigalvarez, M. Kalm, Y. de Pablo, Å. Widestrand, M. Pekna, M. Pekny, The role of GFAP and vimentin in learning and memory, Biol. Chem. 10 (2019).
[107] J.M. Koolhaas, Animal behaviour in the laboratory P. Silverman. Chapman and Hall, London, 1978, 421 pp., pound 15.00, ISBN 412-21580-2, Behav Processes 4 (3) (1979) 279–280.
[108] Z.W. Lu, S. Hayley, A.V. Ravindran, Z. Merali, H. Anisman, Influence of psychosocial, psychogenic and neurogenic stressors on several aspects of immune functioning in mice, Stress 3 (1) (1999) 55–70.
[109] K. Karelina, A.C. DeVries, Modeling social influences on human health, Psychosom Med 73 (1) (2011) 67–74.
[110] A.L. Martin, R.E. Brown, The lonely mouse: verification of a separation-induced model of depression in female mice, Behav Brain Res 207 (1) (2010) 196–207.
[111] D.L. Wallace, M.H. Han, D.L. Graham, T.A. Green, V. Vialou, S.D. Iniguez, J.L. Cao, A. Kirk, S. Chakravarty, A. Kumar, V. Krishnan, R.L. Neve, D.C. Cooper, C.A. Bolanos, M. Barrot, C.A. McClung, E.J. Nestler, CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits, Nat Neurosci 12 (2) (2009) 200–209.
[112] N. Kaushal, D. Nair, D. Gozal, V. Ramesh, Socially isolated mice exhibit a blunted homeostatic sleep response to acute sleep deprivation compared to socially paired mice, Brain Res. 1454 (2012) 65–79.
[113] A. Dranovsky, A.M. Picchini, T. Moadel, A.C. Sisti, A. Yamada, S. Kimura, E.D. Leonardo, R. Hen, Experience dictates stem cell fate in the adult hippocampus, Neuron 70 (5) (2011) 908–923.
[114] J. Liu, J.L. Dupree, M. Gacias, R. Frawley, T. Sikder, P. Naik, P. Casaccia, Clemastine enhances myelination in the prefrontal cortex and rescues behavioral changes in socially isolated mice, J. Neurosci. 36 (3) (2016) 957–962.
[115] E.J. Chesler, S.G. Wilson, W.R. Lariviere, S.L. Rodriguez-Zas, J.S. Mogil, Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive, Neurosci. Biobehav. Rev. 26 (8) (2002) 907–923.
[116] J.C. Crabbe, D. Wahlsten, B.C. Dudek, Genetics of mouse behavior: interactions with laboratory environment, Science 284 (5420) (1999) 1670–1672.
[117] Y.K. Urbach, K.A. Raber, F. Canneva, A.-C. Plank, T. Andreasson, H. Ponten, J. Kullingsjö, H.P. Nguyen, O. Riess, S. von Hörsten, Automated phenotyping and advanced data mining exemplified in rats transgenic for Huntington's disease, J. Neurosci. Methods 234 (2014) 38–53.
[118] N. Wu, F. Wang, Z. Jin, Z. Zhang, L.-K. Wang, C. Zhang, T. Sun, Effects of GABAB receptors in the insula on recognition memory observed with intellicage, Behav. Brain Functions 13 (1) (2017).
[119] L.K. Too, B. Yau, A.G. Baxter, I.S. McGregor, N.H. Hunt, Double deficiency of toll-like receptors 2 and 4 alters long-term neurological sequelae in mice cured of pneumococcal meningitis, Sci. Rep. 9 (1) (2019) 16189.
[120] N.M. Ben Abdallah, L. Slomianka, A.L. Vyssotski, H.P. Lipp, Early age-related changes in adult hippocampal neurogenesis in C57 mice, Neurobiol Aging 31 (1) (2010) 151–161.
[121] R. Akbergenov, S. Duscha, A.K. Fritz, R. Juskeviciene, N. Oishi, K. Schmitt, D. Shcherbakov, Y. Teo, H. Boukari, P. Freihofer, P. Isnard-Petit, B. Oettinghaus, S. Frank, K. Thiam, H. Rehrauer, E. Westhof, J. Schacht, A. Eckert, D. Wolfer, E.C. Böttger, Mutant MRPS5 affects mitoribosomal accuracy and confers

stress-related behavioral alterations, EMBO reports 19 (11) (2018) e46193.
[122] F. Cathomas, M. Stegen, H. Sigrist, L. Schmid, E. Seifritz, M. Gassmann, B. Bettler, C.R. Pryce, Altered emotionality and neuronal excitability in mice lacking KCTD12, an auxiliary subunit of GABAB receptors associated with mood disorders, Translational Psychiatry 5 (2) (2015) e510-e510.
[123] B.P. Festa, M. Berquez, A. Gassama, I. Amrein, H.M. Ismail, M. Samardzija, L. Staiano, A. Luciani, C. Grimm, R.L. Nussbaum, M.A. De Matteis, O.M. Dorchies, L. Scapozza, D.P. Wolfer, O. Devuyst, OCRL deficiency impairs endolysosomal function in a humanized mouse model for Lowe syndrome and Dent disease, Human Mol. Genet. 28 (12) (2019) e1005058.
[124] M. Fischer, V. Cabello, S. Popp, S. Krackow, L. Hommers, J. Deckert, K.-P. Lesch, A.G. Schmitt-Böhrer, Rsk2 knockout affects emotional behavior in the IntelliCage, Behav. Genet. 47 (4) (2017) 434–448.
[125] A. Gumucio, L. Lannfelt, L.N. Nilsson, Lack of exon 10 in the murine tau gene results in mild sensorimotor defects with aging, BMC Neuroscience 14 (1) (2013) 148.
[126] I. Heinla, J. Åhlgren, E. Vasar, V. Voikar, Behavioural characterization of C57BL/6N and BALB/c female mice in social home cage – effect of mixed housing in complex environment, Physiol. Behav. 188 (2018) 32–41.
[127] N.I.W. Ismail, N. Jayabalan, S.M. Mansor, C.P. Müller, M. Muzaimi, Chronic mitragynine (kratom) enhances punishment resistance in natural reward seeking and impairs place learning in mice: mitragynine and cognition, Addiction Biol. 22 (4) (2017) 967–976.
[128] T. Macpherson, M. Morita, Y. Wang, T. Sasaoka, A. Sawa, T. Hikida, Nucleus accumbens dopamine D2-receptor expressing neurons control behavioral flexibility in a place discrimination task in the IntelliCage, Learning & Memory 23 (7) (2016) 359–364.
[129] Z. Mijakowska, K. Łukasiewicz, M. Ziółkowska, M. Lipiński, A. Trąbczyńska, Ż. Matuszek, S. Łęski, K. Radwanska, Autophosphorylation of alpha isoform of calcium/calmodulin-dependent kinase II regulates alcohol addiction-related behaviors: αCaMKII and alcohol addiction, Addiction Biol. 22 (2) (2017) 331–341.
[130] S. Muthuraju, S. Taha, S. Pati, M. Rafique, H. Jaafar, J.M. Abdullah, Normabaric hyperoxia treatment improved locomotor activity of C57BL/6J mice through enhancing dopamine genes following fluid-percussion injury in Striatum, Int. J. Biomed. Sci. 9 (4) (2013) 11.
[131] H. Ogi, K. Itoh, S. Fushiki, Social behavior is perturbed in mice after exposure to bisphenol A: a novel assessment employing an IntelliCage, Brain and Behavior 3 (3) (2013) 223–228.
[132] H. Ogi, K. Itoh, H. Ikegaya, S. Fushiki, Alterations of neurotransmitter norepinephrine and gamma-aminobutyric acid correlate with murine behavioral perturbations related to bisphenol A exposure, Brain and Dev. 37 (8) (2015) 739–746.
[133] P. Pelsőczi, G. Lévay, Effect of scopolamine on mice motor activity, lick behavior and reversal learning in the IntelliCage, Neurochem. Res. 42 (12) (2017) 3597–3602.
[134] A. Raab, S. Popp, K.-P. Lesch, M.J. Lohse, M. Fischer, J. Deckert, L. Hommers, Increased fear learning, spatial learning as well as neophobia in Rgs2 −/− mice: increased fear learning spatial learning and neophobiain Rgs2 −/− mice, Genes, Brain and Behavior 17 (4) (2018) e12420.
[135] D.M. Roccaro-Waldmeyer, F. Girard, D. Milani, E. Vannoni, L. Prétôt, D.P. Wolfer, M.R. Celio, Eliminating the VGlut2-Dependent glutamatergic transmission of parvalbumin-expressing neurons leads to deficits in locomotion and vocalization, decreased pain sensitivity, and increased dominance, Frontiers in Behav. Neurosci. 12 (2018).
[136] B. Schuler, J. Vogel, B. Grenacher, R.A. Jacobs, M. Arras, M. Gassmann, Acute and chronic elevation of erythropoietin in the brain improves exercise performance in mice without inducing erythropoiesis, The FASEB J. 26 (9) (2012) 3884–3890.
[137] D.A. Simmons, N.P. Belichenko, E.C. Ford, S. Semaan, M. Monbureau, S. Aiyaswamy, C.M. Holman, C. Condon, M. Shamloo, S.M. Massa, F.M. Longo, A small molecule p75NTR ligand normalizes signalling and reduces Huntington's disease phenotypes in R6/2 and BACHD mice, Human Mol. Genet. (2016) ddw316.
[138] L.K. Too, K.M. Li, C. Suarna, G.J. Maghzal, R. Stocker, I.S. McGregor, N.H. Hunt, Behavioral and cognitive data in mice with different tryptophan-metabolizing enzymes knocked out, Data in Brief 9 (2016) 275–287.
[139] L.K. Too, I.S. McGregor, A.G. Baxter, N.H. Hunt, Altered behaviour and cognitive function following combined deletion of Toll-like receptors 2 and 4 in mice, Behav. Brain Res. 303 (2016) 1–8.
[140] W. Ujita, A. Kohyama-Koganeya, N. Endo, T. Saito, H. Oyama, Mice lacking a functional NMDA receptor exhibit social subordination in a group-housed environment, The FEBS J. 285 (1) (2018) 188–196.
[141] R.M. van Dijk, F. Wiget, D.P. Wolfer, L. Slomianka, I. Amrein, Consistent within-group covariance of septal and temporal hippocampal neurogenesis with behavioral phenotypes for exploration and memory retention across wild and laboratory small rodents, Behav. Brain Res. (2019) 11.
[142] V. Voikar, G. Colacicco, O. Gruber, E. Vannoni, H.-P. Lipp, D.P. Wolfer, Conditioned response suppression in the IntelliCage: assessment of mouse strain differences and effects of hippocampal and striatal lesions on acquisition and retention of memory, Behav. Brain Res. 213 (2) (2010) 304–312.
[143] J. Zheng, R. Jiang, M. Chen, Z. Maimaitiming, J. Wang, G.J. Anderson, C.D. Vulpe, J.L. Dunaief, H. Chen, Multi-copper ferroxidase–Deficient mice have increased brain Iron concentrations and learning and memory deficits, J. Nutrition 148 (4) (2018) 643–649.
[144] A. Cały, M.A. Śliwińska, M. Ziółkowska, K. Łukasiewicz, R. Pagano, A. Nowacka, M. Borczyk, K. Radwanska, Contribution of PSD-95 protein to reward location memory, bioRxiv (2019).
[145] Z. Harda, J.M. Dzik, M. Nalberczak-Skóra, K. Meyza, K. Łukasiewicz, S. Łęski, K. Radwanska, Autophosphorylation of αCaMKII affects social interactions in mice, Genes, Brain and Behavior 17 (5) (2018) e12457.
[146] S.M. Hölter, L. Garrett, J. Einicke, B. Sperling, P. Dirscherl, A. Zimprich, H. Fuchs, V. Gailus-Durner, M. Hrabě de Angelis, W. Wurst, assessing cognition in mice: assessing cognition in mice, in: J. Auwerx, S.L. Ackerman, S.D. Brown, M.J. Justice, J. Nadeau (Eds.), Current Protocols in Mouse Biology, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2015, pp. 331–358.
[147] G. Maroteaux, T.M. Arefin, L.-A. Harsan, E. Darcq, S. Ben Hamida, B.L. Kieffer, Lack of anticipatory behavior in Gpr88 knockout mice showed by automatized home cage phenotyping, Genes, Brain and Behavior 17 (8) (2018) e12473.
[148] M. Perez-Alcazar, J. Daborg, A. Stokowska, P. Wasling, A. Björefeldt, M. Kalm, H. Zetterberg, K.E. Carlström, K. Blomgren, C.T. Ekdahl, E. Hanse, M. Pekna, Altered cognitive performance and synaptic function in the hippocampus of mice lacking C3, Experimental Neurology 253 (2014) 154–164.
[149] K. Sano, T. Isobe, J. Yang, T.-T. Win-Shwe, M. Yoshikane, S.F. Nakayama, T. Kawashima, G. Suzuki, S. Hashimoto, K. Nohara, C. Tohyama, F. Maekawa, In utero and lactational exposure to acetamiprid induces abnormalities in socio-sexual and anxiety-related behaviors of male mice, Frontiers in Neuroscience 10 (2016).
[150] T. Serchov, I. Schwarz, A. Theiss, L. Sun, A. Holz, M.D. Döbrössy, M.K. Schwarz, C. Normann, K. Biber, D. van Calker, Enhanced adenosine A1 receptor and Homer1a expression in hippocampus modulates the resilience to stress-induced depression-like behavior, Neuropharmacology 162 (2020) 107834.
[151] O. Rudenko, C. Springer, L.J. Skov, A.N. Madsen, L. Hasholt, A. Nørremølle, B. Holst, Ghrelin-mediated improvements in the metabolic phenotype in the R6/2 mouse model of Huntington's disease, J. Neuroendocrinol. 31 (7) (2019).
[152] F. Cathomas, H. Sigrist, L. Schmid, E. Seifritz, M. Gassmann, B. Bettler, C.R. Pryce, Behavioural endophenotypes in mice lacking the auxiliary GABA B receptor subunit KCTD16, Behav. Brain Res. 317 (2017) 393–400.
[153] S. Hardt, C. Fischer, A. Vogel, A. Wilken-Schmitz, I. Tegeder, Distal infraorbital nerve injury: a model for persistent facial pain in mice, PAIN 160 (6) (2019) 1431–1447.
[154] S. Marwari, G.S. Dawe, (R)-fluoxetine enhances cognitive flexibility and hippocampal cell proliferation in mice, J. Psychopharmacol. 32 (4) (2018) 441–457.
[155] S.A. Mohammadi, T.J. Burton, M.J. Christie, α9-nAChR knockout mice exhibit dysregulation of stress responses, affect and reward-related behaviour, Behav. Brain Res. 328 (2017) 105–114.
[156] M. Piechota, M. Korostynski, M. Sikora, S. Golda, J. Dzbek, R. Przewlocki, Common transcriptional effects in the mouse striatum following chronic treatment with heroin and methamphetamine, Genes, Brain and Behavior 11 (4) (2012) 404–414.
[157] P.-S. Welz, V.M. Zinna, A. Symeonidi, K.B. Koronowski, K. Kinouchi, J.G. Smith, I.M. Guillén, A. Castellanos, S. Furrow, F. Aragón, G. Crainiciuc, N. Prats, J.M. Caballero, A. Hidalgo, P. Sassone-Corsi, S.A. Benitah, BMAL1-driven tissue clocks respond independently to light to maintain homeostasis, Cell 177 (6) (2019) 1436–1447 e12.
[158] N.M.-B. Ben Abdallah, R.K. Filipkowski, M. Pruschy, P. Jaholkowski, J. Winkler, L. Kaczmarek, H.-P. Lipp, Impaired long-term memory retention: common denominator for acutely or genetically reduced hippocampal neurogenesis in adult mice, Behav. Brain Res. 252 (2013) 275–286.
[159] M.A. Peltola, J. Kuja-Panula, J. Liuhanen, V. Võikar, P. Piepponen, T. Hiekkalinna, T. Taira, S.E. Lauri, J. Suvisaari, N. Kulesskaya, T. Paunio, H. Rauvala, AMIGO-Kv2.1 potassium channel complex is associated with schizophrenia-related phenotypes, Schizophrenia Bulletin (2015) sbv105.
[160] H. Fröhlich, M.L. Kollmeyer, V.C. Linz, M. Stuhlinger, D. Groneberg, A. Reigl, E. Zizer, A. Friebe, B. Niesler, G. Rappold, Gastrointestinal dysfunction in autism displayed by altered motility and achalasia in *Foxp1* $^{+/-}$ mice, Proc. Nat. Acad. Sci. 116 (44) (2019) 22237–22245.
[161] B. Albuquerque, A. Häussler, E. Vannoni, D.P. Wolfer, I. Tegeder, Learning and memory with neuropathic pain: impact of old age and progranulin deficiency, Frontiers in Behav. Neurosci. 7 (2013).
[162] E. Dere, A. Ronnenberg, B. Tampe, S. Arinrad, M. Schmidt, E. Zeisberg, H. Ehrenreich, Cognitive, emotional and social phenotyping of mice in an observer-independent setting, Neurobiol. Learning and Memory 150 (2018) 136–150.
[163] H. Fuchs, J.A. Aguilar-Pimentel, O.V. Amarie, L. Becker, J. Calzada-Wack, Y.-L. Cho, L. Garrett, S.M. Hölter, M. Irmler, M. Kistler, M. Kraiger, P. Mayer-Kuckuk, K. Moreth, B. Rathkolb, J. Rozman, P. da Silva Buttkus, I. Treise, A. Zimprich, K. Gampe, C. Hutterer, C. Stöger, S. Leuchtenberger, H. Maier, M. Miller, A. Scheideler, M. Wu, J. Beckers, R. Bekeredjian, M. Brielmeier, D.H. Busch, M. Klingenspor, T. Klopstock, M. Ollert, C. Schmidt-Weber, T. Stöger, E. Wolf, W. Wurst, A.Ö. Yildirim, A. Zimmer, V. Gailus-Durner, M. Hrabě de Angelis, Understanding gene functions and disease mechanisms: phenotyping pipelines in the German Mouse Clinic, Behav. Brain Res. 352 (2018) 187–196.
[164] R. Iwata, K. Ohi, Y. Kobayashi, A. Masuda, M. Iwama, Y. Yasuda, H. Yamamori, M. Tanaka, R. Hashimoto, S. Itohara, T. Iwasato, RacGAP α2-Chimaerin function in development adjusts cognitive ability in adulthood, Cell Reports 8 (5) (2014) 1257–1264.
[165] L.R. Jensen, L. Garrett, S.M. Hölter, B. Rathkolb, I. Rácz, T. Adler, C. Prehn, W. Hans, J. Rozman, L. Becker, J.A. Aguilar-Pimentel, O. Puk, K. Moreth, M. Dopatka, D.J. Walther, V. von Bohlen und Halbach, M. Rath, M. Delatycki, B. Bert, H. Fink, K. Blümlein, M. Ralser, A. Van Dijck, F. Kooy, Z. Stark, S. Müller, H. Scherthan, J. Gecz, W. Wurst, E. Wolf, A. Zimmer, M. Klingenspor, J. Graw, T. Klopstock, D. Busch, J. Adamski, H. Fuchs, V. Gailus-Durner, M.H. de Angelis, O. von Bohlen und Halbach, H.-H. Ropers, A.W. Kuss, A mouse model for

intellectual disability caused by mutations in the X-linked 2′-O-methyltransferase Ftsj1 gene, Biochimica et Biophysica Acta (BBA) – Mol. Basis of Dis. 1865 (9) (2019) 2083–2093.
[166] D.J. Koss, L. Robinson, B.D. Drever, K. Plucińska, S. Stoppelkamp, P. Veselcic, G. Riedel, B. Platt, Mutant Tau knock-in mice display frontotemporal dementia relevant behaviour and histopathology, Neurobiol. Dis. 91 (2016) 105–123.
[167] K. Mätlik, V. Võikar, C. Vilenius, N. Kulesskaya, J.-O. Andressoo, Two-fold elevation of endogenous GDNF levels in mice improves motor coordination without causing side-effects, Sci. Reports 8 (1) (2018).
[168] P.R. Netrakanti, B.H. Cooper, E. Dere, G. Poggi, D. Winkler, N. Brose, H. Ehrenreich, Fast cerebellar reflex circuitry requires synaptic vesicle priming by Munc13-3, The Cerebellum 14 (3) (2015) 264–283.
[169] A. Orock, S. Logan, F. Deak, Munc18-1 haploinsufficiency impairs learning and memory by reduced synaptic vesicular release in a model of Ohtahara syndrome, Mol. Cellular Neurosci. 88 (2018) 33–42.
[170] H. Pan, B. Oliveira, G. Saher, E. Dere, D. Tapken, M. Mitjans, J. Seidel, J. Wesolowski, D. Wakhloo, C. Klein-Schmidt, A. Ronnenberg, K. Schwabe, R. Trippe, K. Mätz-Rensing, S. Berghoff, Y. Al-Krinawe, H. Martens, M. Begemann, W. Stöcker, F.-J. Kaup, R. Mischke, S. Boretius, K.-A. Nave, J.K. Krauss, M. Hollmann, F. Lühder, H. Ehrenreich, Uncoupling the widespread occurrence of anti-NMDAR1 autoantibodies from neuropsychiatric disease in a novel autoimmune model, Molecular Psychiatry (2018).
[171] K. Sekiguchi, S. Imamura, T. Yamaguchi, M. Tabuchi, H. Kanno, K. Terawaki, Y. Kase, Y. Ikarashi, Effects of yokukansan and donepezil on learning disturbance and aggressiveness induced by intracerebroventricular injection of amyloid β protein in mice, Phytotherapy Research 25 (4) (2011) 501–507.
[172] D.L. Vyssotski, Heritable compensation of disturbed functionality, Evolocus 13 (2011).
[173] A. Masuda, Y. Kobayashi, S. Itohara, Automated, Long-term Behavioral Assay for Cognitive Functions in Multiple Genetic Models of Alzheimer's Disease, Using IntelliCage, J. Visualized Experiments (138) (2018).
[174] L. de Hoz, D. Gierej, V. Lioudyno, J. Jaworski, M. Blazejczyk, H. Cruces-Solís, A. Beroun, T. Lebitko, T. Nikolaev, E. Knapska, I. Nelken, L. Kaczmarek, Blocking c-Fos expression reveals the role of auditory cortex plasticity in sound frequency discrimination learning, Cerebral Cortex 28 (5) (2018) 1645–1655.
[175] R. d'Isa, S.J. Clapcote, V. Voikar, D.P. Wolfer, K.P. Giese, R. Brambilla, S. Fasano, Mice lacking Ras-GRF1 show contextual fear conditioning but not spatial memory impairments: convergent evidence from two independently generated mouse mutant lines, Front Behav. Neurosci. 5 (2011) 78.
[176] K.H. Aung, C. Kyi-Tha-Thu, K. Sano, K. Nakamura, A. Tanoue, K. Nohara, M. Kakeyama, C. Tohyama, S. Tsukahara, F. Maekawa, Prenatal exposure to arsenic impairs behavioral flexibility and cortical structure in mice, Frontiers in Neurosci. 10 (2016).
[177] S. Hardt, J. Heidler, B. Albuquerque, L. Valek, C. Altmann, A. Wilken-Schmitz, M.K.E. Schäfer, I. Wittig, I. Tegeder, Loss of synaptic zinc transport in progranulin deficient mice may contribute to progranulin-associated psychopathology and chronic pain, Biochimica et Biophysica Acta (BBA) – Mol. Basis of Dis. 1863 (11) (2017) 2727–2745.
[178] J. Ruud, J. Alber, A. Tokarska, L. Engström Ruud, H. Nolte, N. Biglari, R. Lippert, Ä. Lautenschlager, P.E. Cieślak, Ł. Szumiec, M.E. Hess, H.S. Brönneke, M. Krüger, H. Nissbrandt, T. Korotkova, G. Silberberg, J. Rodriguez Parkitna, J.C. Brüning, The fat mass and obesity-associated protein (FTO) regulates locomotor responses to novelty via D2R medium spiny neurons, Cell Reports 27 (11) (2019) 3182–3198 e9.
[179] G. Atlan, A. Terem, N. Peretz-Rivlin, K. Sehrawat, B.J. Gonzales, G. Pozner, G.-i. Tasaka, Y. Goll, R. Refaeli, O. Zviran, B.K. Lim, M. Groysman, I. Goshen, A. Mizrahi, I. Nelken, A. Citri, The claustrum supports resilience to distraction, Current Biol. 28 (17) (2018) 2752–2762 e7.
[180] J. Jabłońska, Ł. Szumiec, P. Zieliński, J.R. Parkitna, Time elapsed between choices in a probabilistic task correlates with repeating the same decision, bioRxiv (2019).
[181] S. Benner, T. Endo, M. Kakeyama, C. Tohyama, Environmental insults in early life and submissiveness later in life in mouse models, Frontiers in Neurosci. 9 (2015).
[182] G. Bergamini, F. Cathomas, S. Auer, H. Sigrist, E. Seifritz, M. Patterson, C. Gabriel, C.R. Pryce, Mouse psychosocial stress reduces motivation and cognitive function in operant reward tests: a model for reward pathology with effects of agomelatine, European Neuropsychopharmacol. 26 (9) (2016) 1448–1464.
[183] A. Beroun, M. Nalberczak-Skóra, Z. Harda, M. Piechota, M. Ziółkowska, A. Cały, R. Pagano, K. Radwanska, Generation of silent synapses in dentate gyrus correlates with development of alcohol addiction, Neuropsychopharmacology 43 (10) (2018) 1989–1999.
[184] J.Y. Holgate, H. Garcia, S. Chatterjee, S.E. Bartlett, Social and environmental enrichment has different effects on ethanol and sucrose consumption in mice, Brain and Behav. 7 (8) (2017) e00767.
[185] M. Koskela, T.P. Piepponen, J.-O. Andressoo, V. Võikar, M. Airavaara, Towards developing a model to study alcohol drinking and craving in female mice housed in automated cages, Behav. Brain Res. 352 (2018) 116–124.
[186] K. Jastrzębska, M. Walczak, P.E. Cieślak, Ł. Szumiec, M. Turbasa, D. Engblom, T. Błasiak, J.R. Parkitna, Loss of NMDA receptors in dopamine neurons leads to the development of affective disorder-like symptoms in mice, Scientific Reports 6 (1) (2016).
[187] U. Skupio, M. Sikora, M. Korostynski, A. Wawrzczak-Bargiela, M. Piechota, J. Ficek, R. Przewlocki, Behavioral and transcriptional patterns of protracted opioid self-administration in mice: patterns of opioid addiction, Addiction Biology 22 (6) (2017) 1802–1816.
[188] S. Alboni, S. Poggini, S. Garofalo, G. Milior, H. El Hajj, C. Lecours, I. Girard, S. Gagnon, S. Boisjoly-Villeneuve, N. Brunello, D.P. Wolfer, C. Limatola, M.-È. Tremblay, L. Maggi, I. Branchi, Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment, Brain, Behavior, and Immunity 58 (2016) 261–271.
[189] C. Ratner, L.J. Skov, Z. Raida, T. Bächler, K. Bellmann-Sickert, C. Le Foll, B. Sivertsen, L.S. Dalbøge, B. Hartmann, A.G. Beck-Sickinger, A.N. Madsen, J. Jelsing, J.J. Holst, T.A. Lutz, Z.B. Andrews, B. Holst, Effects of peripheral neurotensin on appetite regulation and its role in gastric bypass surgery, Endocrinology 157 (9) (2016) 3482–3492.
[190] D.C. Ajonijebu, O. Abboussi, M.V. Mabandla, W.M.U. Daniels, Differential epigenetic changes in the hippocampus and prefrontal cortex of female mice that had free access to cocaine, Metabolic Brain Disease 33 (2) (2018) 411–420.